DETERMINING ALTERNATIVE AND SUSTAINABLE MANAGEMENT STRATEGIES TO MANAGE THE NORTHERN ROOT-KNOT NEMATODE (*MELOIDOGYNE HAPLA*) IN ORNAMENTAL PLANT PRODUCTION FIELDS

By

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ABSTRACT

The United States floriculture industry was valued at $6.43 billion in 2021, with Michigan being the third largest producer, producing 10% of all ornamental plants in the United States. A major constraint to the production of bare-rooted ornamental plants grown in the field are plant-parasitic nematodes. In Michigan, plant-parasitic nematodes cause millions of dollars in economic loss each year in the state's $104.7 billion agriculture industry. In the northern United States and Canada, the northern root-knot nematode, *Meloidogyne hapla*, is the most economically important perennial ornamental pathogen. While this is a known major pathogen of daylily production, one of top commodities in ornamental plant production in Michigan, very little is known about its impact in daylily production fields or how to effectively manage this pest. There are only two main management strategies for *M. hapla* in ornamental plant fields: hot water dips and preplant fumigation, both of which do not control *M. hapla* the entire production cycle and are therefore only semi-effective. Therefore, research was conducted to determine alternative management strategies to manage *M. hapla* in daylily production fields, with the goal to prevent yield loss and exportation rejection, and reduce the economic burden of this pest. Three multi-year field trials at a commercial nursery in Zeeland, MI, and several greenhouse experiments at Michigan State University's Plant Greenhouses, East Lansing, MI, were conducted to test several different management options and combination of management options to find the best new management strategies to control *M. hapla* in ornamental plant fields. The results of these studies demonstrate that there are more effective solutions for *M. hapla* management in ornamental plant field production compared to current practices and highlight three new management options: Indemnify as a soil drench, Majestene 304, and TerraClean 5.0 have been shown to provide the best *M. hapla* management in daylily fields, with a reduction in *M. hapla* population levels by 39.5%, 34.7%, and 28.8%, respectively, compared to the control. Indemnify also reduced the number of galled roots by 80% compared to the control plants, which is considerable and can lead to less fields being quarantined and fewer shipment rejections, significantly increasing the profits of the ornamental plant industry. The Indemnify treatment was additionally shown to have a significant positive effect on plant growth, producing plants with some of the largest overall plant biomass, such as plant heights, shoot weights, crown widths, and, most importantly, yield. Plants where Indemnify was applied as a soil drench always

had higher yields (on average 41.3% higher) compared to the control plants and higher yields (on average 40% higher) compared to Telone II fumigation. These experiments also show that the annual application of treatments throughout the production cycle is crucial and provides significantly better *M. hapla* management compared to current practices, which only focuses on managing nematodes at the beginning of the production cycle. Most importantly, these trials show that there was no impact on plant growth, health, and yield from annual treatment applications. Even though *M. hapla* is well established in these monoculture, long-term ornamental plant fields, a trial determining possible soil suppression showed that natural suppression may not be occurring in ornamental plant fields in Michigan, but more experiments are needed. Two greenhouse trials tested the damage potential and host status of *Hemerocallis* spp. to *M. hapla* and *Paratylenchus* spp., and determined the threshold level of *M. hapla*. These greenhouse experiments show that daylily is an excellent host to *M. hapla*, with a threshold level as low as 13 *M. hapla*/100 cm^3 soil. The data also suggests that even though *M. hapla* affects plant growth, daylily plants may actually be tolerant to *M. hapla;* over the length of the daylily growth cycle, the plants became more tolerant of its feeding and grew to similar sizes of the nematode-free plants. Lastly, daylily was shown to not be a host to *Paratylenchus* spp., and therefore, these nematodes do not need to be included in management decisions. Through the application of the new alternative and more sustainable management strategies described in this dissertation, *M. hapla* can be effectively and efficiently managed in ornamental plant fields leading to a significant advancement in the floriculture industry in Michigan, the northern United States, and Canada.

This dissertation is dedicated to my family and loved ones.
Thank you for always being there for me.

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CHAPTER 1: PLANT-PARASITIC NEMATODES AND THEIR EFFECTS ON ORNAMENTAL PLANTS

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1.1 INTRODUCTION

Plant-parasitic nematodes are a global pathogen and are estimated to cause $173 billion in economic loss to agriculture worldwide with over $13 billion in annual agricultural loss in the United States (Elling, 2013). Plant-parasitic nematodes are known to infect every species of cultivated plants and a large majority of weeds, making them one of the greatest threats to crops worldwide (Handoo, 1998). These pests are microscopic, aquatic roundworms that live in the soil or in plant parts, and feed on plant tissues causing significant yield loss. The damage caused by nematodes can be extensive and are a result of the nematodes using their stylet, a hypodermic-like mouthpart, to puncture plant cells, inject enzymes and hormones, and remove cell contents when feeding. Due to their feeding, diseased plants are generally stunted, chlorotic, wilted, and have reduced and deformed root systems. This leads to reduced yields, reduced winter hardiness, dieback in perennials, and predisposition of the plant to secondary infections by other pathogens (Anwar and Van Gundy, 1989; Handoo, 1998; Phani et al., 2021). Roughly 5 to 10% of all crop losses worldwide are due to plant-parasitic nematodes (Mitiku, 2018).

While much research has been devoted to the effect and management of plant-parasitic nematodes in agricultural crops, one of the less-studied fields is their effect on the ornamental plant industry. This is a huge oversight considering the worldwide ornamental plant industry has an estimated value of $70 billion (Madhavan et al., 2021). The ornamental plant industry is a high demand, high growth industry, and is a valuable export to many countries (Madhavan et al., 2021). The countries with the highest ornamental production are the Netherlands, China, and the United States (Darras, 2020), with the developed countries being the dominant consumers (Adebayo et al., 2020). Cut flowers are one of the top commodities, along with flower bulbs, cut foliage, and bedding plants (Adebayo et al., 2020; Darras, 2020).

In the United States, the floriculture industry was valued at $4.8 billion in 2020 (USDA, 2021) and the environmental horticulture industry, or green industry, which is comprised of nurseries, greenhouses, turfgrass sod producers, landscape design, construction and maintenance firms, and wholesale and retail distribution centers, was valued at $159.6 billion in 2018 (Hall et al., 2020). The United States' largest producers in the ornamental plant industry are Florida, California, Michigan, New Jersey, and Ohio. Florida is by far the largest contributor to the U.S. ornamental plant industry, with a value of $1.14 billion (USDA, 2021). Within the United States' ornamental industry, the highest category of sales went to annual bedding, followed by potted flowering plants, foliage plants, herbaceous perennial plants, propagative materials, and then cut plants and flowers (USDA, 2021). One of the fastest growing aspects in the North American ornamental plant industry is specialty cut flower production due to a huge consumer demand (Darras, 2020).

One of the most economically important ornamental plants in the United States is daylilies, *Hemerocallis* spp. Daylilies are a perennial, herbaceous monocot and are widely cultivated across the majority of North America. Native to eastern Asia, daylilies were brought to North America by European immigrants in the 1800s and have over 98,000 registered cultivars in the American Hemerocallis Society (American Daylily Society, 2023; Emmitt and Buck, 2017). Daylilies derive their common name and are famous for having their flowers bloom for only one day. Tolerant to a wide range of climates and soil types, daylilies are also low maintenance, heat- and drought-tolerant making them one of the most popular ornamental perennial plants in landscapes and gardens (Mosonyi et al., 2019; Gulia et al., 2009; Gatlin, 1999). One of the reasons they are tolerant to a wide range of environmental conditions is their roots. Daylilies have two types of roots: thick, rhizomatous roots and fine, fibrous roots. The rhizomatous roots can store large amounts of water and nutrients while the fibrous roots are how the plant absorbs water and nutrients (Gulia et al., 2009).

Daylilies are asexually propagated and planted in the field as bare-rooted propagules. They are grown in the field for two to three years until they contain several crowns that can be divided into smaller plants. After their production cycle in the field, their foliage is cut off and they are machine harvested, with the majority of their roots also removed. Each plant is then split up by dividing its crown into smaller plants called eyes, or clumps of the rhizomes; each eye can be sold as a new daylily plant. Other ways plants are grown in the ornamental plant industry are by tissue culturing and the propagation of corms, plugs, bulbs, and rhizomes. Plant-parasitic nematodes, pathogens, diseases, viruses, and insects can be easily spread through this propagation of plant parts or on plant material from field harvested plants. One of the most important pathogens of this list are plant-parasitic nematodes. Within the ornamental plant industry, there are many nematodes that affect ornamental plants worldwide (Table 1), with the main genera being *Meloidogyne* spp., *Aphelenchoides* spp., *Paratylenchus* spp., *Pratylenchus* spp., *Helicotylenchus* spp., *Radopholus* spp., *Xiphinema* spp., *Trichodorus* spp., *Paratrichodorus* spp., *Rotylenchulus* spp., and *Longidorus* spp. (Taylor, 1972; Bala and Hosein, 1996; Handoo, 1998; Inserra et al., 1998; Mitiku, 2018).

Table 1.1. Plant-parasitic nematodes found in floricultural crops worldwide and their referenced papers.

Host Scientific Name	Host Common Name	Nematode Species	Reference
Hippeastrum sp.	Amaryllis	*Pratylenchus coffeae, P. hippeastri*	Crow and Duncan, 2018; Inserra et al., 2007; Pinochet and Duarte, 1986
Anthurium sp.	Anthurium	*Aphelenchoides fragariae, A. ritzemabosi, Helicotylenchus dihystera, Meloidogyne incognita, M. javanica, Paratylenchus minutus, P. shenzhenensis, Pratylenchus coffeae, Radopholus similis, Rotylenchulus reniformis*	Bala and Hosein, 1996; Kohl, 2011; Phani et al., 2021; Sipes and Myers, 2018; Wang et al., 2013
Didiscus caeruleus	Blue lace flower	*Meloidogyne* spp.	Wang and McSorley, 2005
Buxus sp.	Boxwood	*Meloidogyne incognita*, *Mesocriconema* spp.; *Pratylenchus* spp.; *P. vulnus, Rotylenchus buxophilus*	Eisenback, 2018; Lehman, 1984
Caladium sp.	Caladium	*Meloidogyne* spp.	Gu et al., 2022
Calendula officinalis	Calendula	*Aphelenchoides ritzemabosi, Meloidogyne* spp.	Kohl, 2011; Wheeler et al., 2018
Dianthus caryophyllus	Carnation	*Criconema xenoplex, Ditylenchus myceliophagus, Helicotylenchus digonicus, H. dihystera, H. pseudorobustus, Heterodera daverti, Longidorus elongatus, Meloidogyne incognita, M. javanica, Pratylenchus neglectus, P.*	Borgohain, 2016; Chandel et al., 2010; Deimi et al., 2008; Lung et al., 1997; Phani et al., 2021; Taylor, 1972

Table 1.1. (cont'd)

Dianthus caryophyllus	Carnation	*thornei, Rotylenchulus reniformis*, *Xiphinema diversicaudatum*	
Chrysanthemum sp.	Chrysanthemum	*Aphelenchoides besseyi, A. fragariae, A. ritzemabosi, Ditylenchus myceliophagus*, *Helicotylenchus digonicus, H. pseudorobustus, H. vulgaris, Meloidogyne incognita, M. javanica, Pratylenchus neglectus, P. penetrans, P. thornei, Rotylenchulus reniformis*	Borgohain, 2016; Christie and Birchfield, 1958; Deimi et al., 2008; Handoo, 1998; Khan, 2015; Kohl, 2011; Mitiku, 2018; Yamamoto and Toida, 1995
Dahlia sp.	Dahlia	*Aphelenchoides ritzemabosi*	Khan, 2015
Hemerocallis sp.	Daylily	*Aphelenchoides ritzemabosi, Helicotylenchus dihystera, Meloidogyne arenaria, M. hapla, M. incognita, Paratrichodorus* spp., *Paratylenchus* spp., *Pratylenchus* spp., *Rotylenchulus reniformis, Scutellonema brachyurus*	Howland et al., 2022; Inserra et al., 1995; Kohl, 2011; LaMondia, 1996; Ye, 2018
Xanthosoma sp.	Elephant's ears	*Meloidogyne* spp., *Rotylenchulus reniformis, Pratylenchus coffeae*	Jatala and Bridge, 1990
Gardenia jasminoides	Gardenia	*Aphelenchoides fragariae, Meloidogyne* spp.	Crow and Duncan, 2018; Kohl, 2011
Geranium sp.	Geranium	*Aphelenchoides fragariae*	Kohl, 2011
Gerbera jamesonii	Gerbera	*Aphelenchoides fragariae, Longidorus elongatus, Meloidogyne incognita, M. javanica, Pratylenchus coffeae, Rotylenchulus reniformis*	Borgohain, 2016; Kohl, 2011; Phani et al., 2021
Alpinia sp.	Ginger lily	*Criconemella onoensis*, *Helicotylenchus dihystera, H. pseudorobustus, Meloidogyne incognita, Peltamigratus* spp., *Pratylenchus* spp.,	Bala and Hosein, 1996

Table 1.1. (cont'd)

Alpinia sp.	Ginger lily	*Rotylenchulus reniformis, Tylenchorhynchus annulatus*	
Gladiolus grandiflorus	Gladiolus	*Aphelenchoides besseyi, Dityle Helicotylenchus crenacauda*, *H. digonicus, H. pseudorobustus, Meloidogyne incognita, Pratylenchus coffeae, P. thornei, Xiphinema americanum nchus myceliophagus*,	Borgohain, 2016; Deimi et al., 2008; Taylor, 1972
Alcea rosea	Hollyhock	*Meloidogyne incognita*	Khan et al., 2005; Wheeler et al., 2018
Hosta sp.	Hosta	*Aphelenchoides* spp., *A. fragariae*	Jagdale and Grewal, 2006; Kohl, 2011
Hydrangea macrophylla	Hydrangea	*Aphelenchoides besseyi, A. fragariae, Ditylenchus dipsaci*	Kohl, 2011; Ye, 2018
Iris sp.	Iris	*Aphelenchoides fragariae, A. ritzemabosi, Helicotylenchus digonicus, H. pseudorobustus, H. vulgaris, Pratylenchus neglectus, P. thornei*	Deimi et al., 2008; Kohl, 2011
Ipomoea purpurea	Morning Glory	*Aphelenchoides ritzemabosi, Meloidogyne* spp.	Kohl, 2011; Wheeler et al., 2018
Consolida ajacis	Larkspur	*Meloidogyne* spp.	Wang and McSorley, 2005
Lavandula angustifolia	Lavender	*Aphelenchoides ritzemabosi, Meloidogyne hapla*	Kohl, 2011; LaMondia, 1995
Lilium sp.	Lily	*Pratylenchus penetrans*	Chitambar et al., 2018
Eustoma grandiflorum	Lisianthus	*Meloidogyne* spp.	Wang and McSorley, 2005
Polystichum adiantiforme	Leatherleaf fern	*Pratylenchus neglectus*	Rhoades, 1968
Heliconia	Lobster Claw	*Helicotylenchus dihystera, Meloidogyne incognita, Pratylenchus* spp., *Rotylenchulus reniformis,*	Bala and Hosein, 1996

Table 1.1. (cont'd)

Petunia hybrida	Petunia	*Aphelenchoides ritzemabosi, Meloidogyne incognita*	Khan et al., 2005; Kohl, 2011; Wheeler et al., 2018
Papaver rhoeas	Poppy	*Meloidogyne incognita*	Khan et al., 2005
Rosa sp.	Rose	*Aphelenchoides* spp., *Criconemella* spp., *Ditylenchus myceliophagus, Helicotylenchus crenacauda, H. pseudorobustus, H. vulgaris, Meloidogyne hapla, M. incognita, M. javanica, Pratylenchus* spp., *P. neglectus,*	Deimi et al., 2008; Phani et al., 2021; Yamamoto and Toida, 1995
Rosa sp.	Rose	*P. thornei, Rotylenchulus reniformis*	
Ficus sp.	Rubber plant	*Pratylenchus coffeae*	Pinochet and Duarte, 1986
Tulipa sp.	Tulip	*Aphelenchoides ritzemabosi, Ditylenchus dipsaci, Helicotylenchus pseudorobustus, Meloidogyne incognita, Paratrichodorus* spp., *Pratylenchus neglectus, P. thornei, Trichodorus* spp.	Borgohain, 2016; Deimi et al., 2008; Kohl, 2011; Madhavan et al., 2021
Antirrhinum majus	Snapdragon	*Aphelenchoides ritzemabosi, Helicotylenchus pseudorobustus, Meloidogyne* spp.	Kohl, 2011; Wang and McSorley, 2005; Wheeler et al., 2018
Helianthus sp.	Sunflower	*Aphelenchoides ritzemabosi, Meloidogyne incognita, Paratylenchus projectus, Pratylenchus thornei*	Khan, 2015; Kohl, 2011; Loof, 1975; Rashad et al., 2011
Zinnia sp.	Zinnia	*Aphelenchoides besseyi, A. fragariae, A. ritzemabosi, Meloidogyne* spp.	Khan, 2015; Kohl, 2011; Wheeler et al., 2018

1.1.1 Root-Knot Nematodes

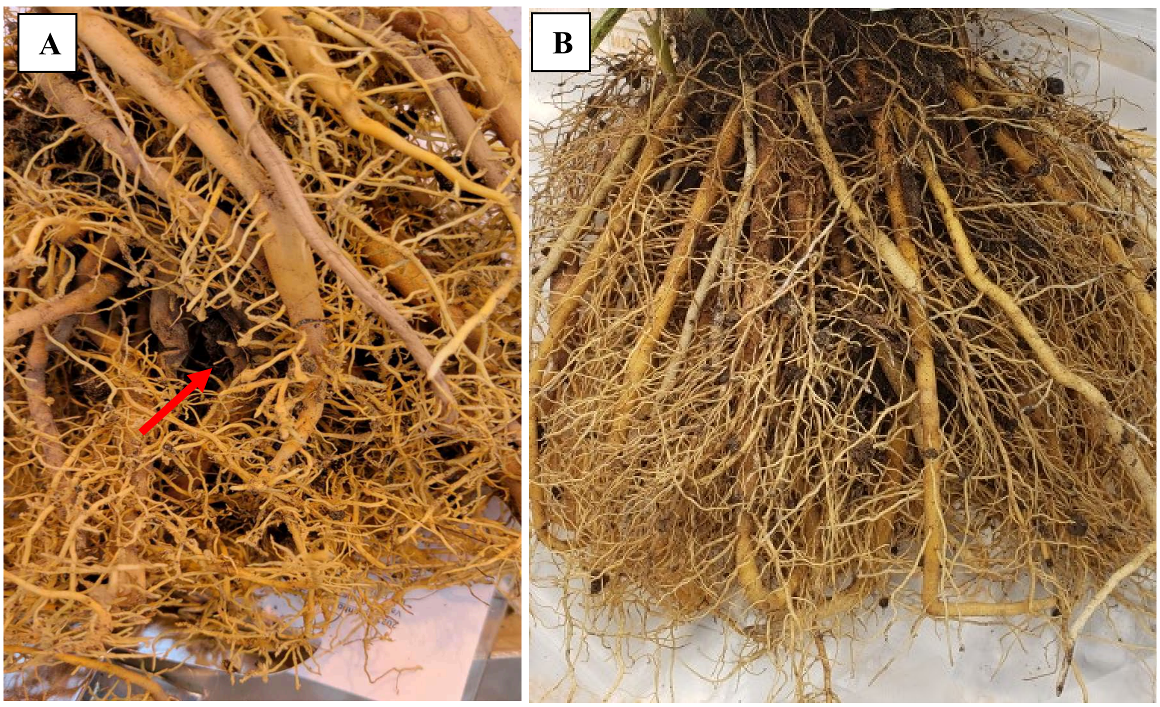
The most economically devasting and important plant-parasitic nematode is the root-knot nematode, *Meloidogyne* spp., due to its worldwide distribution and host range of over 3,000 plant species (Abad et al., 2003). There are over 100 described *Meloidogyne* species resulting in these nematodes infecting almost every agricultural crop and most weeds (Hussey and Janssen, 2002; Elling, 2013). Root-knot nematodes (Fig. 1A) are sedentary endoparasites remaining stationary inside the roots of a host plant with the plant growing around them to form galls (Taylor and Sasser, 1978). Small galls with usually a single nematode occur on young feeder roots and larger galls can be a consequence of multiple infections at the same location. In agricultural cultivated fields, there are four major *Meloidogyne* spp. that account for 95% of all root-knot infestations: *M. incognita* (Kofoid and White, 1919) Chitwood, 1949, *M. hapla* Chitwood, 1949, *M. javanica* (Treub, 1885) Chitwood, 1949, and *M. arenaria* (Neal, 1889) Chitwood, 1949 (Hussey and Janssen, 2002). Among root-knot nematode species, the northern root-knot nematode, *Meloidogyne hapla*, is the most important perennial ornamental pathogen in the northern United States and Canada (LaMondia, 1996), whereas in the southern United States, a variety of tropical root-knot nematode species infect ornamentals (Brito et al., 2010).

Figure 1.1. Light micrograph of *Meloidogyne hapla* second-stage juvenile extracted from a daylily field at a commercial nursery in Michigan.

Symptoms of root-knot nematode infection include galled and stunted roots (Fig. 1B), plants that wilt easily and are stunted, and have poor vigor and other symptoms common in nutrient deficiencies like chlorosis. Additionally, yield can be dramatically reduced in bare-rooted ornamentals that are grown in the field leading to over 20% yield loss (Lindberg et al., 2018). In cut foliage crops, root-knot nematode infection can lead to slower regrowth of leaves and stems (Baidoo et al., 2017). Lastly, their characteristic galls on the roots further reduce marketability and profits, and can prohibit plant shipments from being sold and distributed; one gall from root-knot nematodes can reject an entire shipment (Poley et al., 2018). In herbaceous perennial plants that have large, fleshy roots, the northern root-knot nematode galls can easily be inconspicuous and hard to notice leading to the accidental spread of these nematodes in exports.

Current management strategies are limited for managing this important pest, and typically consist of hot water dip treatments (Powell and Riedel, 1978; Inserra et al., 1995; Daughtrey and Benson, 2005), where propagules are dipped in hot water for a period of time to kill nematodes; and preplant soil fumigation, which is extremely costly and toxic to the environment. Since 2005 when methyl bromide was banned due to its ozone depleting capabilities, there has been a shift to move away from harmful fumigants to less damaging controls such as biological nematicides and organic amendments (Zasada et al., 2010).

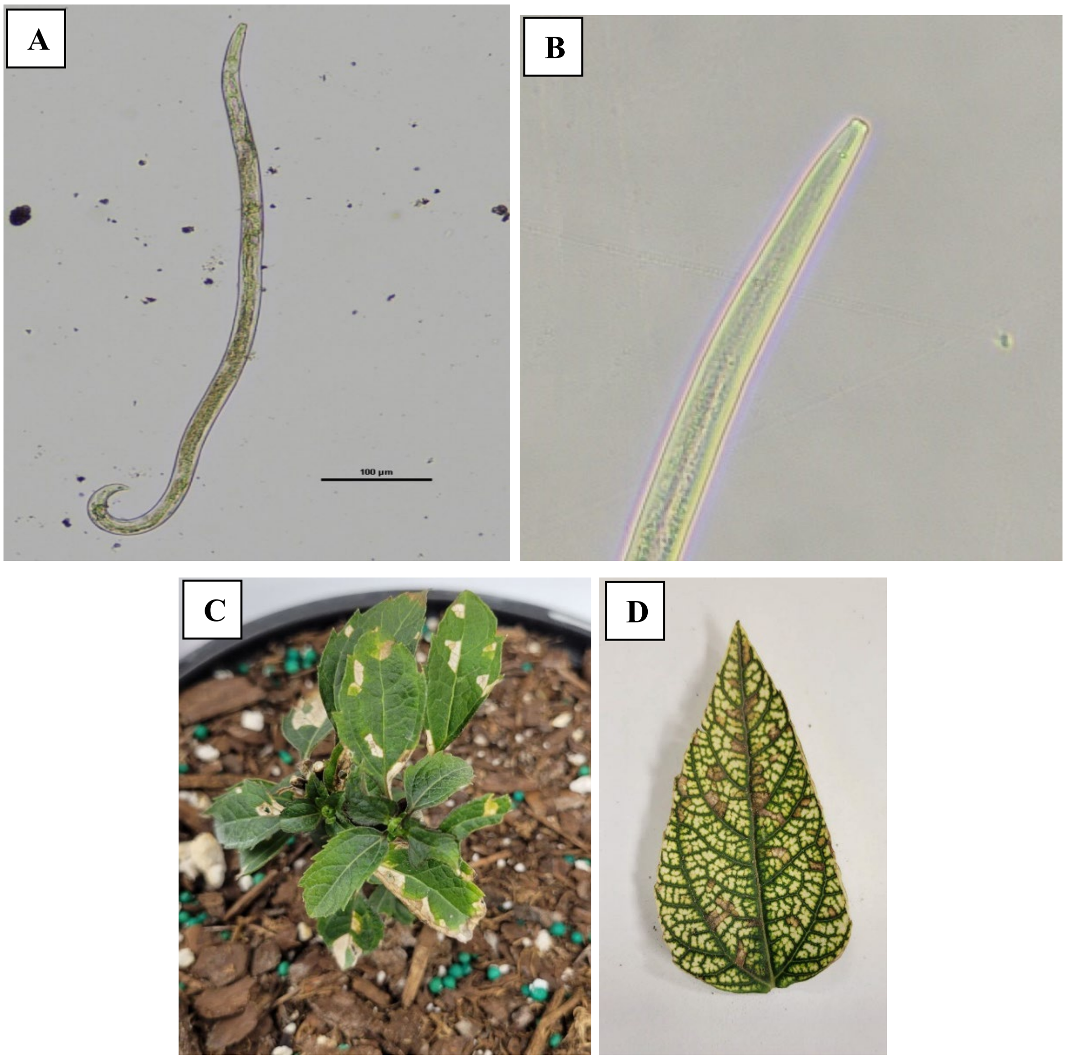
Figure 1.2. Daylily roots taken from a commercial ornamental plant field showing galling and stunting due to *Meloidogyne hapla* infection (A) compared to healthy roots (B). The red arrow points to the galls on the root system.



1.1.2 Foliar Nematodes

Foliar nematodes, *Aphelenchoides* spp. (Fig. 2A, B), are aerial nematodes that have a wide host range of over 700 plant species, including many ornamental plants like hosta and chrysanthemum (Table 1) (Handoo, 1998; Jagdale and Grewal, 2006; Kohl, 2011; Mitiku, 2018). The three main species of economic importance to the ornamental industry are *A. fragariae* (Ritzema Bos, 1891) Christie, 1942, *A. ritzemabosi* (Schwartz, 1911) Steiner and Buhrer, 1932, and *A. besseyi* Christie, 1942 (Kohl, 2011). Foliar nematodes are primarily endoparasites that feed predominately inside the leaves, but depending on environmental conditions, foliar nematodes can also feed externally on the leaves and flower buds of some plants. These nematodes mostly overwinter in the soil and migrate up the outside of plant stems in films of water to the leaves. They are spread easily through infected plant seeds and are commonly splashed around in water via rain or overhead irrigation from infected plants to plants nearby. Symptoms from foliar nematode feeding are chlorotic, angular lesions on the leaves that run parallel to major leaf veins and can eventually become necrotic if feeding persists (Fig. 2C, D). This can lead to leaves having a dry, tattered appearance and severe infection can kill the whole leaf causing defoliation (Jagdale and Grewal, 2006; Kohl, 2011; Mitiku, 2018; Phani et al., 2021).

Figure 1.3. Light micrograph of an adult male (A) and head (B) of *Aphelenchoides* spp. extracted from *Heliopsis* spp. leaves. Angular lesions (C, D) on the leaves of two varieties of *Heliopsis* spp. infected with *Aphelenchoides* spp.



Management of foliar nematodes can be difficult since *Aphelenchoides* spp. can survive in infested dried leaf debris and in dormant plant crowns for many years, and they are easily splashed around (Jagdale and Grewal, 2006; El-Saadony et al., 2021). However, there are various methods that can be employed to manage these nematodes. Current management methods include using drip irrigation instead of overhead watering and sanitation of tools, pots, and soil that come in contact with infected plants by heat treating them via baking or steaming (Mitiku, 2018). Other treatments include hot water drenches on infected plants, their leaves, and dormant plant materials (Jagdale and Grewal, 2004; Kohl, 2011; Mitiku, 2018), the use of humic acid derived from manure (Nagachandrabose and Baidoo, 2021), and plant resistance in some plant species (Kohl, 2011; Mitiku, 2018; El-Saadony et al., 2021).

1.1.3 Virus Vectoring Nematodes

There are two different types of nematode transmitted viruses based on the viruses' particle shape: NEPO viruses (nematode polyhedral viruses) and TOBRA viruses (tubular or rod-shaped viruses) (Taylor, 1972). These viruses are transmitted through nematode feeding on plant tissues. NEPO viruses are transmitted by *Xiphinema* (dagger) and *Longidorus* (needle) nematodes; both ectoparasitic nematodes are large and slender. Their host range consists of perennial and woody plants, grapevines, orchids, and small fruits (Taylor, 1972; Handoo, 1998). Main NEPO viruses for ornamental plants, such as geraniums, petunia, and tulips, are tomato ringspot virus (ToRSV), tobacco ringspot virus (TRSV), Arabis mosaic virus (ArMV), and tomato black ring (TBRV) virus (Engelmann and Hamacher, 2008). While there are numerous NEPO viruses, most of which are species dependent, general symptoms include pronounced ring and mosaic lined patterns, chlorotic flecking, mottling, leaf curling and necrosis, stunted plants, and general plant decline.

TOBRA viruses are transmitted by *Trichodorus* and *Paratrichodorus* species (stubby root nematodes), which are thick nematodes with a curved stylet. Similar to dagger and needle nematodes, they feed externally at the root tip, causing the root tips to swell and become stubby (Taylor, 1972). The main viruses are Tobacco mosaic virus (TMV), Tobacco rattle virus (TRV), Pea early-browning virus (PEBV), and Pepper ringspot virus (PepRSV) which can affect plants like petunias, tulips, and lilies (Taylor, 1972; Handoo, 1998; Engelmann and Hamacher, 2008; Macfarlane, 2010; Khan, 2015). General symptoms of TOBRA viruses include chlorotic and necrotic spots or strips on leaves, mosaic, mottling, light or dark colored flecks on flower petals, oval lesions, flower malformation, and stunted plants (Engelmann and Hamacher, 2008; Madhavan et al., 2021).

Management of these nematodes to prevent virus spread and transmission include using only virus-free planting material, hygienic measures and disinfection of tools and equipment, thermotherapy, destroying virus infected plants to help prevent the spread of inoculum, and regular testing of plant stocks (Taylor, 1972; Engelmann and Hamacher, 2008; Adaskaveg and Caprile, 2009; Madhavan et al., 2021). Weed control is especially important since they can serve as alternative hosts for nematodes and can act as virus reservoirs. For example, dandelion, *Taraxacum officinale*, and other broadleaf weeds can harbor viruses, and nematodes can feed on them, obtain the virus, and then transmit it to ornamental plants in the same field (Adaskaveg and Caprile, 2009). Fumigants for controlling the nematode vector can be used preplanting as a management option. Additionally, using both nematode and virus resistant cultivars can be a very effective management option. For example, in tulips, there are several TBV-resistant cultivars such as 'Cantata', 'Juan', 'Madame Lefeber', and 'Princeps' (Madhavan et al., 2021). While these management methods can be effective, there is a need for more research, especially since plant propagules can also harbor viruses leading to their spread.

1.1.4 Other Plant-Parasitic Nematodes

There are many other plant-parasitic nematodes that can infect and severely damage ornamental plants (Table 1). Some of the other main plant-parasitic nematodes are *Pratylenchus* spp., or lesion nematode, which is a migratory endoparasite that has a host range of over 400 plant species, including amaryllis (Christie and Birchfield, 1958; Nong and Weber, 1965), ferns (Rhoades, 1968), and foliage plants such as rubber plants (Pinochet and Duarte, 1986). Symptoms of lesion nematode feeding in ornamental plants has been reported to be stunting of roots and shoots, chlorotic foliage, foliage discoloration, wilting, root swelling, brown lesions, destruction of the entire root systems, and severe yield loss (Christie and Birchfield, 1958; Rhoades, 1968; Mitiku, 2018). Symptoms can also include necrotic lesions on plant roots which can lead to secondary infections from bacteria and fungi causing disease complexes. *Radopholus similis* (Cobb, 1893) Thorne, 1949, the burrowing nematode, is another migratory endoparasite that is an important pest in anthurium and black pepper (Haegeman et al., 2010; Khan, 2015), with over 350 additional hosts. Feeding from *R. similis* in the roots and stems causes root decay and rot, severe plant stunting and chlorosis, and can cause plant dieback and death (Borgohain, 2016; Wang et al., 2016). In anthurium rhizomes, *R. similis* can also disrupt the vascular bundles causing necrosis and anatomical alterations in the roots (Vovlas et al., 2003), or they can fail to produce any noticeable symptoms in anthurium canes, which are used to plant new fields, leading to the accidental spread of this nematode (Sipes and Myers, 2018).

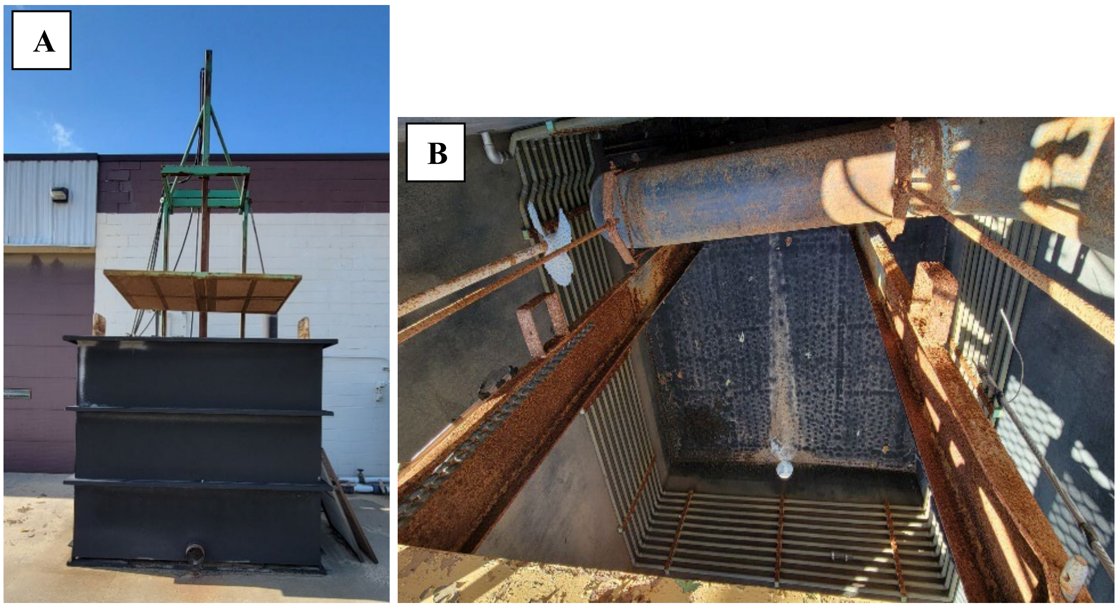
Rotylenchulus reniformis Linford and Oliveira, 1940, the reniform nematode, is a semi-endoparasitic nematode that partially penetrates plant roots. These nematodes produce no plant symptoms in some ornamental plants like daylily (Inserra et al., 1998), but they can be devasting parasites to crops such as cotton (Koenning et al., 2004). *Helicotylenchus* spp., the spiral nematode, is a migratory ectoparasite that has a large host range, but very little research has been conducted on this nematode in ornamental plants, except for *Rotylenchus buxophilus* Golden, 1956, the boxwood spiral nematode, which causes slow decline in boxwood plants (Lehman, 1984; Eisenback, 2018). Another migratory ectoparasite, *Paratylenchus* spp., or pin nematode, is also commonly found in ornamental plant fields, such as daylily (Howland et al., 2022), but to date, very little research has been conducted on this nematode species in ornamental plants except on *P. shenzhenensis* Wang, 2013 on anthurium (Wang et al., 2016). Symptoms of *Paratylenchus* spp. infection includes stunting, low quality plants, and decreased yields.

1.2 PLANT-PARASITIC NEMATODE MANAGEMENT AND GAPS

Plant-parasitic nematodes cost the ornamental plant industry millions of dollars due to their symptoms, yield loss, and plant exportation rejection as described in this review. Current management strategies are not very effective in ornamental production (LaMondia, 1996), making management of plant-parasitic nematodes in ornamental plants a challenging task. This is especially true due to the loss of effective but environmentally degrading pesticides, propagation and movement of asymptomatic plants, and lack of general knowledge.

For certain ornamental plants, especially those grown exclusively in the field for several years like daylily, iris, and hosta, current management strategies consist of hot water treatments (drenches and dips) and preplant fumigation. Hot water drenches are used to disinfect plants from pests such as insects and plant-parasitic nematodes, such as the stem and bulb nematode, *Ditylenchus dipsaci* (Kuhn, 1857) Filipjev, 1936, *A. fragariae*, and *R. similis*. Plant material that is typically treated with hot water are bare-rooted plants, tubers, runners, and dormant plant material, where the material is dipped in a large tank of hot water (Fig. 3A, B) then cooled down in secondary water tanks. Hot water dips can kill other endoparasitic nematodes, such as *Meloidogyne* spp., but can have little to no impact on ectoparasitic nematodes that live in the soil and remain outside the roots; planting heat-treated plants into soil already infected with ectoparasitic nematodes will not prevent infection. While effectively killing plant-parasitic nematodes, both hot water drenches and dips can cause plant mortality and reduced vigor, germination, and growth in propagules (Rhoades, 1964; Jagdale and Grewal, 2004; Tsang et al., 2004; Howland et al., 2022). Dipping the plants in nematicides, instead of just hot water, as shown by Howland et al. (2022), is an alternative management strategy that shows potential. In that trial, daylily plugs were dipped in a fluopyram solution before planting; those plants had significantly higher biomass with moderate control of root-knot nematodes compared to the control and Telone II fumigation. However, testing of nematicide chemicals as a preplant dip or post-harvest treatment in perennials needs further investigation.

Figure 1.4. Hot water dipping tank (A) and the interior of the tank (B) at a commercial nursery in Michigan.



The second main management strategy is soil fumigation. For decades, soil fumigation has been a main tactic to control plant-parasitic nematodes in agricultural systems throughout the United States (Zasada et al., 2010). In most crops such as vegetable crops, preplant fumigation is the dominant management strategy for root-knot nematodes (Hajihassani, 2018). Pre-plant soil fumigation is very effective at managing *Meloidogyne* spp., but in production systems longer than a year, such as daylilies, fumigation controls nematodes only in the first year; it does not control nematodes throughout the entire production cycle. In Michigan ornamental nurseries, fumigation only provides 60-70% control of root-knot nematodes with a cost of half a billion dollars/year. It provides inadequate control since soil fumigation can only be applied before planting, and not while plants are in the ground since it is phytotoxic (Noling, 2008), thus leading to unsatisfactory control of nematodes after several years of plant growth, especially in the last year of the plant's production cycle. In addition, most fumigant nematicides are high-risk/high-cost products (Zasada et al., 2010), so a management system or a non-phytotoxic product that can be applied throughout the growing cycle is needed. There are several novel nematicides, including fluensulfone, tioxazafen, and fluopyram (Phani et al., 2021; Howland et al., 2022) that show promising control, and new chemical products continue to improve while having minimal environmental impact (Daughtrey and Benson, 2005).

With an estimated 85,000–99,000 ornamental plant species and their wild relatives worldwide (Long et al., 2018), host status screenings of ornamental plants, and their respective numerous varieties, are an important component of plant-parasitic nematode management. However, this is an understudied part in this industry. For example, there are over 83,000 registered cultivars of daylily, yet only a few varieties have actually been tested as hosts to *M. hapla*. Similarly, *R. similis* can contaminate daylily if the plants are grown in the field in conjunction with another host (Inserra et al., 1998), but there is no research on *R. similis* infection and impact in daylily. Additional host status screenings of the most economically important ornamental plants are needed to determine resistant, susceptible, and tolerant varieties; this information can be used to determine which varieties can be planted in nematode contaminated fields to help prevent yield loss.

Plant resistance is another under-reported aspect, yet it is a highly effective and inexpensive management strategy to control plant-parasitic nematodesin agricultural crops (Hajihassani et al., 2018). Some species of plants are genetically resistant to certain nematodes, such as resistance to some *Aphelenchoides* spp. on hosta, but the extent of plant resistance is largely unknown and focuses more on agricultural crops. Protocols to assess resistance have been established in certain plant species, such as on *Aphelenchoides* spp. in hosta (Zhen et al., 2012), but this is not available in most ornamental plants.

An important aspect of all pathogen management is integrated pest management (IPM), which is a cornerstone of ornamental plant and nursery crop production (Daughtrey and Benson, 2005). Various methods should be used in conjunction with each other to achieve high plant-parasitic nematode control, such as cultural practices, chemical control, clean stock programs, sanitation measures, periodic rotation, and plant resistance. Additionally, biocontrols are becoming a more sustainable management strategy that can be applied to ornamental plants. Examples include using nematode-pathogenic fungi that can parasitize plant-parasitic nematodes of all life stages such as eggs, juveniles, females, and adults, such as *Purpureocillium lilacinum* (Baidoo et al., 2017); these products can even be applied to perennial plants to suppress plant-parasitic nematode population levels (Crow, 2014). The use of commercial products that contain the bacteria *Bacillus firmus* and *Pasteuria penetrans* have been found to be promising as well (Topalović et al., 2020; Phani et al., 2021). However, in a lot of ornamental plants, such as caladium, there is no information available on whether these biological nematicides are effective against plant-parasitic nematodes (Gu et al., 2022). Efficacy trials on these biocontrol agents should be implemented to determine their effects on nematodes, other soil-borne diseases, and overall soil health.

Environmentally sustainable management strategies of plant-parasitic nematodes can be achieved. Pesticides are the still number one management strategy (El-Saadony et al., 2021), although alternative nematode management methods including biocontrol, biological nematicides, thermotherapy, and other cultural practices show promising management in the ornamental plant industry (Khan et al., 2005; Crow, 2014; Desaeger et al., 2020; Howland et al., 2022).

1.3 OUTLOOK AND FUTURE DIRECTIONS

There are many other plant-parasitic nematodes whose economic and damage potential remain unknown in this field. For instance, there are only four main *Meloidogyne* species but there are over 100 described species and some of these lesser known 'minor' species, such as *M. enterolobii* Yang and Eisenback, 1983 and *M. mayaguensis* Rammah and Hirschmann, 1988 are emerging major problems in agriculture and can parasitize ornamental plants (Brito et al., 2007; Elling, 2013). Rarely are these plant-parasitic nematodes reported on in the ornamental plant industry. Only recently has more research been conducted with the majority being first reports, hosts status trials, and testing new nematicides' effectiveness in controlling these pests. Surveys, identification, and ecological studies, such as infection behavior and overwintering survival, of some of the less common plant-parasitic nematodes can be an important advancement in controlling these pests in the ornamental plant industry.

Other directions ornamental plant management should go are improving plant breeding techniques to include plant resistance to nematodes. Plant resistance is an efficient tool for controlling nematodes and the development of resistant varieties can result in reduced yield loss and increased profits in all agricultural industries (Boerma and Hussey, 1992). However, with the wide range of plant-parasitic nematodes and the main focus being on agricultural crops and not ornamental plants, plant resistance to the most important nematode species described here needs to be studied further, along with the identification of new molecular markers for resistance genes in this field. Similarly, molecular identification techniques need to be improved with potentially new primers developed for less common nematode species. Then high-yielding, profitable cultivars can be developed to provide growers with consistently effective nematode management.

Since plant-parasitic nematodes can easily be spread through asymptotic plants, the development of diagnostic tools to detect and subsequently restrict their movement is crucial to preventing their further distribution. PCR-based diagnostic assays do exist for some nematodes, such as *A. fragariae* (McCuiston et al., 2007), *D. dipsaci* (Marek et al., 2005), and *R. similis* (Krishna and Eapen, 2019). However, these diagnostic assays do not exist for most nematodes and are species specific; they do not work for intraspecies within a genus. For instance, the PCR-based diagnostic assay developed for *A. fragariae* by McCuiston et al. (2007), does not work on *A. besseyi* or *A. ritzemabosi* since these assays only use species-specific primers. Development of real-time diagnostic assays for more plant-parasitic nematodes are much needed to detect if nematodes are present in plant exports. This will especially be more important with climate change increasing the spatial distribution and seasonal variation of plant-parasitic nematodes.

In conclusion, limited research has investigated the effect of plant-parasitic nematodes in ornamental plants, probably due to the fact that they are not a food crop. However, considering how fast the ornamental industry is growing and the increasing demand for ornamental flowers and plants, more research needs to be conducted on finding new management options, increasing plant resistance, and better understanding nematode epidemiology. Controlling these nematodes can help prevent their spread through exports and can help reduce yield loss worldwide. Therefore, the focus of this PhD dissertation is to determine alternative management strategies that are sustainable to manage the northern root-knot nematode, *M. hapla*, in ornamental plant fields in Michigan, with a focus on the production of bare-rooted daylily, *Hemerocallis* spp., one of the major components of Michigan's ornamental plant industry. At the conclusion this PhD dissertation, the results from this novel research study will add tremendous value and knowledge to the field of nematology and to the northern United States' and Canadian ornamental plant nurseries. Plant-parasitic nematodes will then be managed in an effective and sustainable way in daylily production fields and yield will significantly increase reducing the economic impact these plant-parasitic nematodes have on the floriculture industry.

1.4 DISSERTATION OBJECTIVES

The goal of this dissertation is to determine sustainable management strategies for the northern root-knot nematode, *Meloidogyne hapla*, in Michigan ornamental plant production fields, specifically *Hemerocallis* spp. fields, to prevent yield loss and crop rejection, and reduce the economic burden of this pest.

Objective I: Determine alternative management strategies to control M. hapla in Hemerocallis spp. production fields.

This objective was accomplished through the application of different chemical nematicides, bio-nematicides, and composts to determine their efficacy in controlling root-knot nematodes in *Hemerocallis* spp. fields to reduce yield loss and increase their marketability. The hypotheses of this objective were that some of the management strategies and new nematicides will provide better control of *M. hapla*, especially in the second and third year of daylily production, without causing phytotoxicity or plant death compared to current management strategies. Also, the number of galls on the daylily root systems will be reduced causing an increase in profit from less shipments getting rejected. This objective was first conducted in the field, via three field trials, then a multi-year greenhouse trial was conducted to test the top management strategies from these field trials. All field research was conducted at Walter's Gardens in Zeeland, Michigan. The greenhouse trial was conducted in the Applied Nematology Lab's Plant Greenhouse at Michigan State University in East Lansing, Michigan.

Objective II: Evaluate the host status of M. hapla and Paratylenchus spp. on Hemerocallis spp. production.

This objective was achieved through the conduction of two replicated greenhouse trials. The hypothesis of this objective was that both of these nematodes parasitize *Hemerocallis* spp., further reducing yield losses in the ornamental industry. All greenhouse trials were conducted in the Applied Nematology Lab's Plant Greenhouse at Michigan State University in East Lansing, Michigan.

Objective III: Determine the production impact and action thresholds of M. hapla and Paratylenchus spp. on Hemerocallis spp. production.

This objective was accomplished through the conduction of two replicated greenhouse trials and the three field trials stated in Objective I. The hypotheses of this objective were that very low population levels of *M. hapla* will significantly impact *Hemerocallis* spp. plant growth and yield. Also, *Hemerocallis* spp. plants can tolerate moderate to high levels of *Paratylenchus* spp. before an impact on yield is detected, since pin nematodes usually only cause economic damage at very high population levels (Ghaderi, 2019). All field research was conducted at Walter's Gardens in Zeeland, Michigan. All greenhouse trials were conducted in the Applied Nematology Lab's Plant Greenhouse at Michigan State University in East Lansing, Michigan.

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CHAPTER 2: ALTERNATIVE MANAGEMENT STRATEGIES AND IMPACT OF THE NORTHERN ROOT-KNOT NEMATODE (*MELOIDOGYNE HAPLA*) IN DAYLILY (*HEMEROCALLIS* SPP.) PRODUCTION

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2.1 INTRODUCTION

The most economically devasting and important plant-parasitic nematode is the root-knot nematode, *Meloidogyne* spp., due to its worldwide distribution and host range of over 2,000 plant species (Agrios, 2005; Baidoo et al., 2017; Jones et al., 2013). These nematodes can infect almost every agricultural crop and most weeds (Hussey and Janssen, 2002). Plant-parasitic nematodes are microscopic, aquatic roundworms that live in the soil and feed on plant roots causing significant yield loss. Root-knot nematodes are sedentary endoparasites remaining stationary inside the roots of a host plant where galls form around them (Taylor and Sasser, 1978). These characteristic galls are visible on the outside of the roots and can prevent the sale and distribution of some agricultural crops, such as ornamental plants.

In the United States, the floriculture industry was valued at $4.8 billion in 2020 (USDA, 2021). Michigan is the third largest producer in the floriculture industry and a major component of that industry is the production of bare-rooted daylily, *Hemerocallis* spp., with an economic value of $16.8 million in 2020 (USDA, 2021). Daylilies are a perennial, herbaceous monocot and are widely cultivated across the majority of North America. They are one of the most popular and important ornamental perennial plants in landscapes and gardens (Gatlin, 1999; Mosonyi et al., 2019). The production of clean plant material can be a challenge for bare-rooted daylily plants grown under field conditions due to plant-parasitic nematodes, especially the northern root-knot nematode, *Meloidogyne hapla* (Benson and Barker, 1985; Lindberg et al., 2018). *Meloidogyne hapla* is the most economically important perennial ornamental pathogen in the northern United States and Canada (LaMondia, 1996).

Daylily plants are grown in the field for up to 3 years and then are commercially propagated by dividing the crown, which consists of many individual plants called eyes (Gulia et al., 2009). Usually, plants are shipped and planted mostly without roots, but root-knot nematodes can still be present in the bulb (Wesemael et al., 2011). High populations of *Meloidogyne* spp. can result in root decay, severely stunted plants, and plant death (Inserra et al., 1995; Powell and Riedel, 1978). *Meloidogyne hapla* are responsible for over 20% yield loss in Michigan daylily production (Lindberg et al., 2018). Additionally, their visible symptoms on the roots further reduce marketability and profits by prohibiting plant sales (Lindberg et al., 2018) since there is a zero-tolerance policy that can lead to the rejection of an entire shipment.

Nematodes are difficult to manage and current management strategies for *M. hapla* in ornamental field production are limited. One of the main management methods is a hot water dip treatment (Daughtrey and Benson, 2005; Inserra et al., 1995; Powell and Riedel, 1978), where daylily rootstocks are 'dipped' in hot water then rapidly cooled. While this method can kill plant-parasitic nematodes, it can also cause up to 50% mortality of the propagules and drastically reduce vigor further causing yield loss (Poley et al., 2018). The other main management strategy is preplant soil fumigation. In Michigan's ornamental nurseries, fumigation provides 60 to 70% control of *M. hapla*, but in production systems longer than one year, such as daylily, fumigation is only effective in the first year as fumigants are phytotoxic and cannot be applied to soil with live plants (Mueller, 2001). Both current management strategies focus on managing nematodes in the field only at the beginning of the daylily production cycle and lose efficacy in the long-term (LaMondia, 1996). This emphasizes the need for efficacious management options of *M. hapla* in ornamental daylily throughout the entire production cycle.

In addition to developing new alternative management strategies for *M. hapla*, there is a need to determine the exact damage potential these pests cause to the floriculture industry. Despite the estimated scale of economic losses by this nematode to the ornamental industry, this system remains understudied (but see Inserra et al., 1998). In particular, research on *M. hapla* and other plant-parasitic nematodes in daylily in Michigan has been completely lacking; consequently, their exact impacts on plant growth are largely unknown. Understanding how different nematode population pressures affect daylily yield and plant performance will fill an important knowledge gap for the floriculture industry.

With these problems facing the ornamental industry, the aims of this study were 1) establish effective management strategies for *M. hapla* in bare-root *Hemerocallis* production through the entire production cycle to produce nematode-free plants and, 2) evaluate the damage impact of *M. hapla* on *Hemerocallis* spp. in a greenhouse experiment with varying rates of inoculum. The results of these studies will be used to develop long-term and effective management recommendations for *M. hapla* with the overall goal of reducing the application of fumigants, reducing plant mortality, and increasing profitability for bare-root ornamentals.

2.2 METHODOLOGIES

2.2.1 Field Trial

A 3-year field trial was conducted at a commercial ornamental nursery in Zeeland, MI. The trial was established in the spring of 2018 and terminated in the fall of 2020. In 2018, a field with a known root-knot nematode infestation was selected for planting *Hemerocallis* spp. cv. 'Going Bananas'; the soil type was Chelsea loamy fine sand (Web Soil Survey, 2020). Prior to planting in the spring, the field was divided into two sections: half was fumigated with Telone II (1,3-dichloropropene; Corteva, Wilmington, DE) and the other half was left unfumigated. The section of the field that was not fumigated was further divided into subplots to evaluate alternative treatments to manage *M. hapla* during all three years of the daylily production cycle.

Eleven treatments were selected to test alternative management strategies to control *M. hapla* in *Hemerocallis* spp. production fields (Table 2.1). Treatments were Indemnify nematicide used both as a plant 'dip' and soil drench application, three compost manures, Advanced Ag bionematicide, AzaGuard bionematicide, Majestene 304 and 305 bionematicides, TerraClean 5.0 nematicide, and an untreated control. These treatments were chosen in part because they can be applied while the plant is in the ground throughout the production cycle. Product performance was compared to the untreated control and a positive control, Telone II.

Each treatment had six replicates that were arranged in a complete randomized block design in the field. Treatments were assigned to each plot using Agriculture Research Manager software (Gylling Data Management Inc., Brookings, SD). The field was divided into 66 plots (3.2 m x 1.1 m) which consisted of 36 plants arranged 6 rows wide by 6 rows deep. Drench applications were applied using 11.4 L watering cans and poured in furrow in the planting row of each respective plot; treatments were added to the watering can before being applied. The Indemnify dip application had daylily plants submerged for 8 min in an Indemnify solution before planting, and the composts were raked into the topsoil (Table 2.1). Immediately after all treatments were applied, daylily plants were planted into each plot according to industry standards. Treatments were applied at the same rate in the spring of each year for all three years of the field trial except for TerraClean 5.0 since it is phytotoxic and could injure the plants if applied at full strength; therefore, it was applied at a half rate to each respective plot in years 2 and 3 (Table 2.1).

Table 2.1. Characteristics and application rates of the treatments applied in the *Hemerocallis* spp. field trial, 2018-2020, at a commercial ornamental nursery in Zeeland, Michigan, to manage *Meloidogyne hapla* during a three-year daylily production cycle.

Treatments	Manufacturer	Active Ingredient	Rate	2018	2019	2020
Indemnify Root Dip	Bayer Environmental Science	Fluopyram	227.7 ppm	Daylily submerged for 8 min	Drench	Drench
Indemnify Drench	Bayer Environmental Science	Fluopyram	41.11 L/ha	Drench	Drench	Drench
Compost 1: Dairy Doo®	Morgan Composting Inc.	Composted dairy cow manure with spelt hulls	0.46 t/ha	Raked in Topsoil	Raked in Topsoil	Raked in Topsoil
Compost 2: Prescription Blend	Morgan Composting Inc.	Composted dairy cow manure with wood ash	0.46 t/ha	Raked in Topsoil	Raked in Topsoil	Raked in Topsoil
Compost 3: 101 Starter Blend	Morgan Composting Inc.	High carbon potting soil	0.46 t/ha	Raked in Topsoil	Raked in Topsoil	Raked in Topsoil
Advanced Ag	Advanced Agriculture Services, LLC	Humic acid/stinging nettle fumigant mixture	122.53 L/ha	Drench	Drench	Drench
AzaGuard	BioSafe Systems	Neem oil	0.27 L/ha	Drench	Drench	Drench
Majestene 304	Marrone Bio Innovations	*Chromobacterium subtsugae*	0.25 t/ha	Drench	Drench	Drench
Majestene 305	Marrone Bio Innovations	*Burkholderia rinojensis* strain A396	1103 L/ha	Drench	Drench	Drench
TerraClean 5.0	BioSafe Systems	Hydrogen peroxide	Full: 41.56 L/ha Half: 20.78 L/ha	Drench – Full Rate	Drench – Half Rate	Drench – Half Rate

Table 2.1. (cont'd)

Untreated Control	--	Untreated Control	--	No Treatment	No Treatment	No Treatment

At the time of the initial treatment applications in the spring of 2018, each plot was soil sampled. Soil samples were composite samples of 10 soil cores randomly taken in the root zone in each plot using a soil probe (25.4 cm by 2.54 cm). Soil samples were also taken at midseason in July and in the fall in October. Plant-parasitic nematodes were extracted from the soil according to standard sucrose centrifugal-flotation methods with slight modifications (Barker et al., 1969; Ingham, 1994; Jenkins, 1964). Briefly, each sample was thoroughly homogenized and a 100 cm^3 soil subsample was processed by mixing with water and then pouring over nested 250-µm and 25-µm sieves with the nematodes retained on the bottom 25-µm sieve. After repeating this process three times, the contents of the 25-µm sieve were centrifuged with a 40% sucrose solution. Then, each sample was poured over a 25-µm sieve and rinsed for 20 s to remove the sucrose from the nematodes. The final contents of the 25-µm sieve were enumerated on an inverted microscope; all plant-parasitic nematodes were counted.

Root samples and plant height measurements from each plot were also taken at midseason in July. Plant heights were recorded using a measuring stick from three plants/plot. Root samples were taken by using a shovel to gather a subsample of fine roots from three plants in each plot; root samples were kept cool until processing. One gram of each root sample was then processed and stained with acid fuchsin stain (Byrd et al., 1983). After the roots were stained and treated with an acidified glycerin destaining solution, the roots were then placed in gridded petri dishes for enumeration. All life stages of *M. hapla* were counted.

In 2019 and 2020, the same soil and root sampling and plant height measurements were taken at the same timings as in 2018. In October 2020, the field trial was terminated. Soil samples were collected as described above and final plant height measurements were taken. Three plants from each plot were dug up to take further growth parameters, such as shoot and root fresh weights (g), crown width (cm), the number of eyes, and yield. A gall rating was not taken due to the difficulty of seeing the small *M. hapla* galls on the field plants. Yield was calculated by determining the number of industry standard ratings of Grade 1 (G1) offshoots for each individual plant.

2.2.2 Greenhouse Experiments

A replicated greenhouse trial was conducted at Michigan State University's Plant Greenhouses, East Lansing, MI to determine the production impact and gain an understanding of damage potentials for *M. hapla* in *Hemerocallis* spp. production. Experiments were conducted in 2021 and 2022, from May until October to mirror the seasonal growth of field daylily plants. In the spring of both years, nursery-grade bare-rooted G1 *Hemerocallis* spp. cv. 'Going Bananas' plants (Walters Gardens, Zeeland, MI) were potted into a 1:1 mix of steam-pasteurized sandy loam soil:sand in 3.7 L pots. The newly potted plants were left to grow for two weeks and then each plant was inoculated with various rates of *M. hapla*. *Meloidogyne hapla* originally collected from a daylily field in Zeeland, MI, and reared on tomato (*Lycopersicon esculentum* Mill. cv. 'Rutgers') was used as inoculum. Inoculum was collected from six-month-old tomato roots according to standard practices using a 10% NaOCl solution (Byrd et al., 1972; Hussey and Barker, 1973; Jenkins, 1964). The *M. hapla* inoculum rates were: 0 (control), 500, 3,000, 5,000, and 10,000 eggs/plant (Baidoo et al., 2017; Bernard and Witte, 1987; Wang et al., 2016). Plants were inoculated by aliquoting the inoculum into four, 5-cm deep holes in the soil around the base of the plant. The experiment composed of eight replicates of each inoculation rate that were arranged in a randomized complete block design in the greenhouse.

Plants were kept at a 16h:8h light:dark photoperiod at 26°C and fertilized weekly (15 ml/7.6 L, Peters' Professional 20-10-20 N-P-K, ICL Specialty Fertilizers, Dublin, OH). Biweekly plant evaluations were recorded to measure plant growth, including plant height (cm), number of eyes, number of buds, and number of scapes. We additionally measured width diameter (cm) N-S and E-W to generate a growth index (Krug et al., 2010).

At the end of the experiment each October, individual plants were destructively harvested to obtain shoot and root fresh weights (g). Additional measurements taken were final plant height and diameter measurements, number of eyes, number of buds, number of scapes, and a *M. hapla* gall rating on a scale of 0 to 5 where 0 = 0 galls, 1 = 1 to 2 galls, 2 = 3 to 10 galls, 3 = 11 to 30 galls, 4 = 31 to 100 galls, and 5 = > 100 galls per root system (Taylor and Sasser, 1978). Lastly, the final population of *M. hapla* was determined for each plant by extracting *M. hapla* eggs from the entire root system (Byrd et al., 1972; Hussey and Barker, 1973; Jenkins, 1964). Briefly, each root system was gently rinsed free of excess soil, placed in a 10% NaOCl solution and shaken vigorously for 4 min, then poured over a set of nested 90-µm and 25-µm sieves. The egg solution was then centrifuged with a 40% sucrose solution to separate the eggs from the soil. Finally, each sample was stained with acid fuchsin to facilitate counting, and eggs were enumerated under an inverted microscope. A reproductive factor (RF) [RF= final nematode population/initial nematode population (Oostenbrink, 1966)] was calculated to help quantify the effect of varying rates of nematodes have on daylily plants. A RF value > 1 indicates that the plant is a good host while a RF value < 1 indicates a poor host.

2.2.3 Statistical Analyses

Data from the field and greenhouse trials were analyzed in R 4.0.3 (R Core Team, 2020). Data distribution was assessed before analysis and a $\log_{10}(x + 1)$ transformation was used to meet normality assumptions, if needed. Analysis of variance (ANOVA) was used followed by means separation to determine if there was a significant difference among the treatments for plant-parasitic nematodes and plant data. Repeated measures analysis using linear regression was also conducted on the biweekly plant evaluations taken both years of the greenhouse trials, such as the growth index measurements, number of eyes, number of buds, and number of scapes. Tukey's honest significance difference test ($P \leq 0.05$) was used to determine differences among treatments in the 'emmeans' package in R (Lenth, 2019).

2.3 RESULTS

2.3.1 Field Trial

Meloidogyne hapla field population levels varied throughout the duration of the trial ($P > 0.05$). Population levels ranged from 0 to 18 *M. hapla*/100 cm^3 soil in the field trial across all three years (data not shown). In 2018, average soil population levels ranged from 0 to 0.83 *M. hapla*/100 cm^3 soil. In 2019, population levels were from 0.13 to 0.63 *M. hapla*/100 cm^3 soil, and in 2020, field population levels ranged from 0.18 to 1.41 *M. hapla*/100 cm^3 soil. Across all three years, the TerraClean 5.0 treatments numerically had the lowest *M. hapla* population densities, with the Indemnify root dip treatments and Compost 3: 101 Starter treatments having the highest mean *M. hapla* population densities. All treatments performed better than the control except the Indemnify root dip treatments and Compost 3: 101 Starter treatments.

Additional plant-parasitic nematodes were found in the field trial, including pin, *Paratylenchus* spp., lesion, *Pratylenchus* spp., and stubby-root nematodes, *Paratrichodorus* spp. *Pratylenchus* spp. and *Paratrichodorus* spp. were found at low densities (< 5 nematodes/100 cm^3 soil) across all years and plots and therefore were not analyzed. *Paratylenchus* spp. population levels in the field were higher and ranged from 0 to 1,368 nematodes/100 cm^3 soil and varied throughout the duration of the field trial ($P < 0.001$). Across all three years, the control plots had the highest *Paratylenchus* spp. population levels with an average of 33.84 *Paratylenchus* spp./100 cm^3 soil; the Indemnify soil drench treatment had the lowest population level of 2.36 *Paratylenchus* spp./100 cm^3 soil. The Telone II fumigated plot had the second highest overall population density of 33.28 *Paratylenchus* spp./100 cm^3 soil in the field.

According to the root analysis (Figure 2.1), the number of *M. hapla*/1 g of roots were significantly different by treatment in 2018 ($P < 0.001$) and 2020 ($P = 0.002$) but not in 2019 ($P = 0.146$), with averaged values ranged from 0 to 54.10 *M. hapla*/1 g of roots. In 2019, the population levels were lower compared to the other two years, while 2020 had the highest population levels. In 2018, the control had the highest *M. hapla*/1 g of roots compared to every treatment except Compost 1: Dairy Doo®. In 2020, Majestene 305 had the highest *M. hapla*/1 g of roots while Compost 2: Prescription Blend had the lowest. Combining the data across all three years, Majestene 304 resulted in the lowest number of *M. hapla* inside the roots with 8.95 *M. hapla*/1 g of roots ($P = 0.037$). The control plots had the second highest level of *M. hapla* found within the roots across all three years of 14.16 *M. hapla*/1 g of roots, while Majestene 305 had the highest at 19.92 *M. hapla*/1 g of roots. Plant heights also varied significantly across treatments in every year except in 2020 (Figure 2.2); heights ranged from 19.60 to 65.69 cm. Compared to the control, in both 2018 and 2019, the fumigated plants had the lowest plant heights and the Indemnify soil drench treatments had the highest ($P \leq 0.05$).

Figure 2.1. Mean ± SEM midseason *Meloidogyne hapla*/1 g of *Hemerocallis* spp. root for each treatment (N=8) in the field trial at midseason in July in 2018, 2019, and 2020. Asterisks indicate significant differences from the control within the same year according to Tukey's HSD ($P \leq 0.05$).

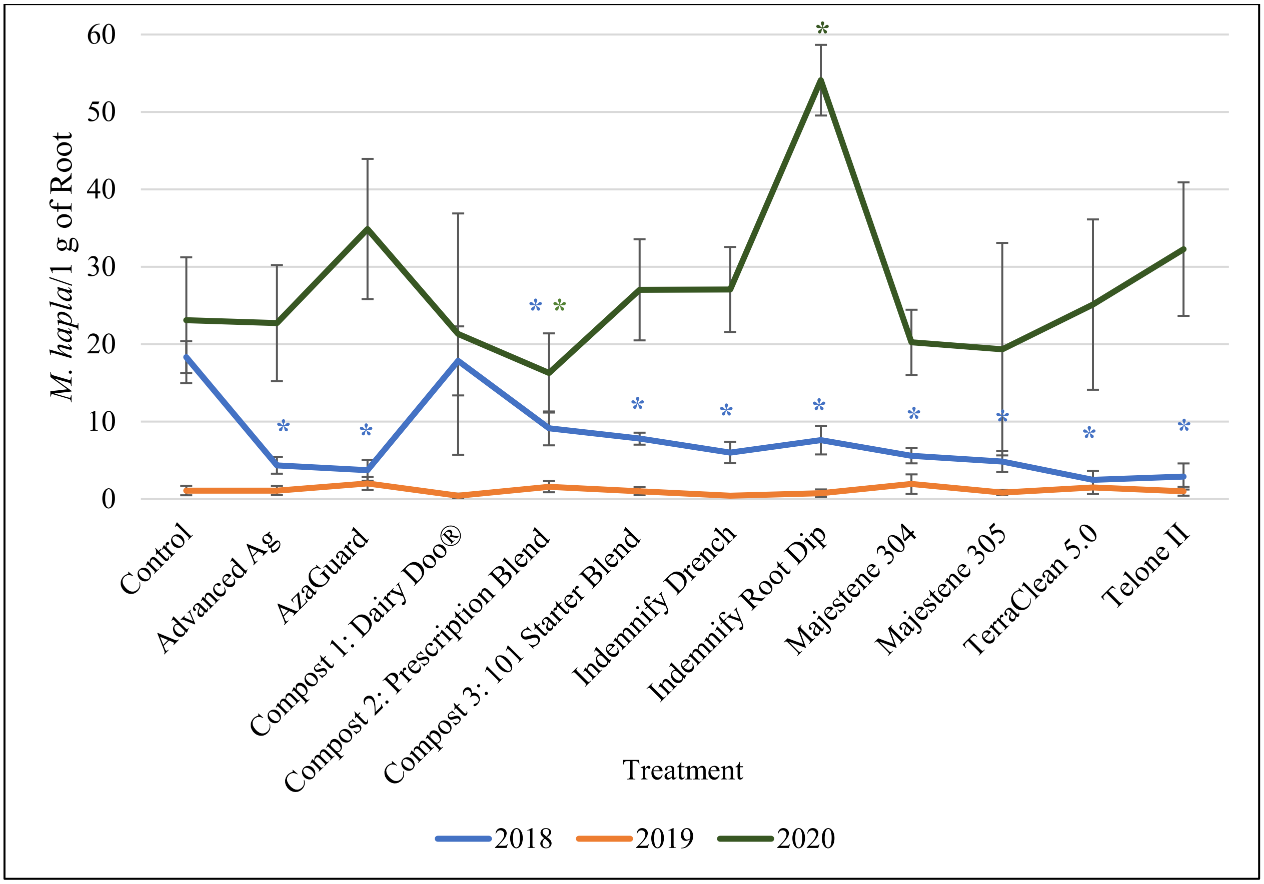
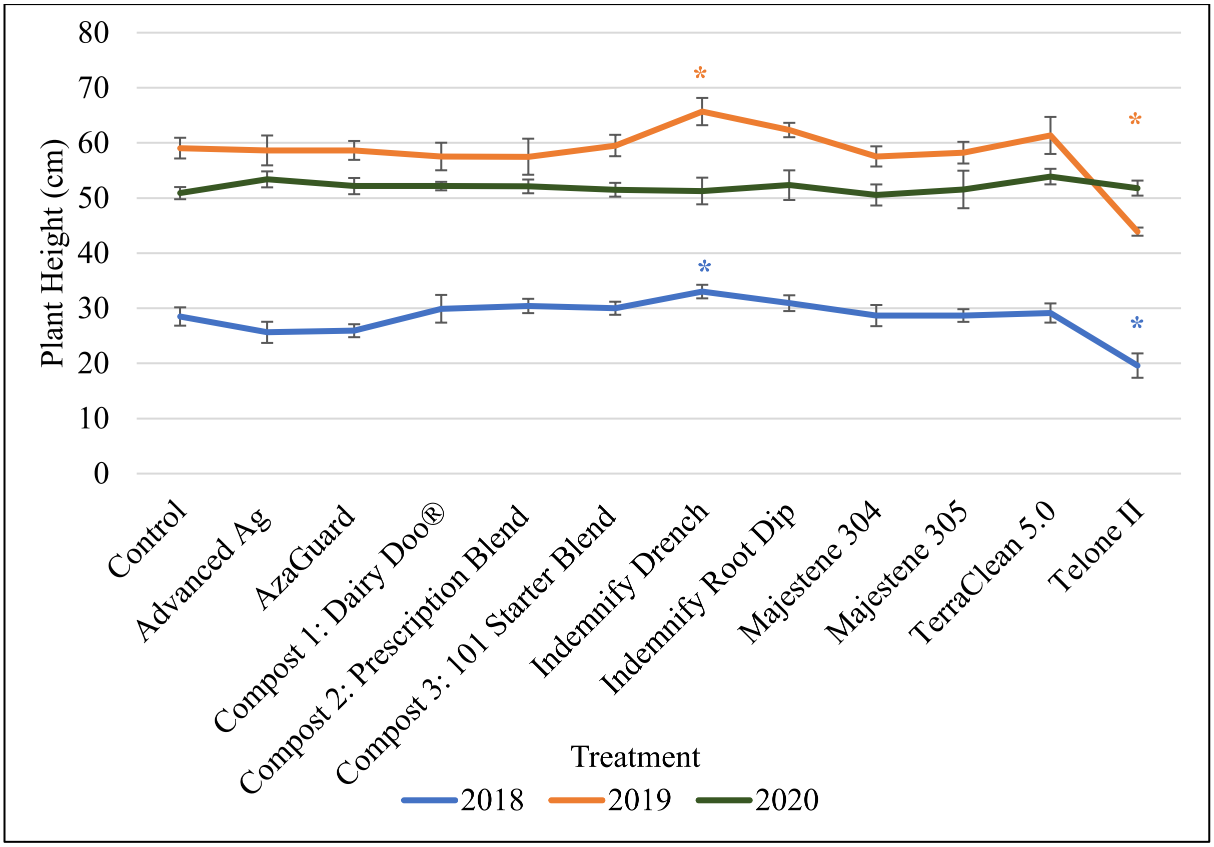


Figure 2.2. Mid-summer mean ± SEM *Hemerocallis* spp. plant height (cm) for each treatment taken in the field trial in the midseason (N=8) in July in 2018, 2019, and 2020. Asterisks indicate significant differences from the control within the same year according to Tukey's HSD ($P \leq 0.05$).



When the experiment was terminated in October 2020, shoot and root fresh weights (g), crown width (cm), the number of eyes, final plant heights (cm), and yield as number of G1s per plant were taken from three plants/plot (Table 2.2). The Compost 3: 101 Starter Blend treatment resulted in the smallest shoot weights while the Telone II fumigated plants had the highest ($P < 0.001$). However, the Telone II fumigated plants had the smallest root system ($P < 0.001$), while the Indemnify soil drench plots had the largest root system. Plants in the Compost 1: Dairy Doo® treatment had the smallest crowns compared to both the Indemnify root dip and drench treatments, though not significantly. The number of eyes/plant differed ($P < 0.001$) with Compost 2: Prescription Blend having 32% less eyes/plant compared to the control; the highest number of eyes was in the Indemnify soil drench treatment which was 29% higher than the control plants. Differences were also observed among final plant height measurements with the

Telone II fumigated plants having the shortest heights by 47% compared to Indemnify soil drench treatment which had the highest (*P* < 0.001). Looking at yield data (G1), the Indemnify soil drench plants had 60% more G1 plants compared to the lowest treatment, Compost 1: Dairy Doo® (*P* < 0.001), with the control plants having a 31% lower yield than the Indemnify soil drench plants.

Table 2.2. Final mean *Hemerocallis* spp. measurements (N=6) of fresh shoot and root weights (g), crown width (cm), number of eyes/plant, plant heights (cm), and Grade 1 (yield) at the end of the three-year field trial in October 2020. Means followed by the same letter within a column are not significantly different according to Tukey's honestly significant difference test ($P \leq 0.05$).

Treatment	**Shoot Weight (g)**	**Root Weight (g)**	**Crown Width (cm)**	**No. of Eyes/Plant**	**Plant Height (cm)**	**Grade 1**
Control	117.17 cd	1,044.28 ab	128.33 a	11.33 bc	49.11 ab	3.10 bc
Advanced Ag	99.06 d	1,009.50 ab	127.78 a	9.44 bc	46.72 bc	2.32 c
AzaGuard	103.22 d	940.28 ab	119.44 a	10.39 bc	46.89 bc	2.50 bc
Compost 1: Dairy Doo®	90.89 d	947.28 ab	117.78 a	9.83 bc	45.22 c	2.28 c
Compost 2: Prescription Blend	91.22 d	864.67 bc	128.33 a	8.61 c	48.83 ab	2.33 c
Compost 3: 101 Starter Blend	87.67 d	935.44 ab	127.78 a	9.67 bc	48.28 ab	2.44 bc
Indemnify Drench	228.67 a	1,300.56 a	138.33 a	14.56 a	62.06 a	4.33 a
Indemnify Root Dip	165.44 bc	913.28 ab	138.33 a	13.00 ab	57.83 ab	3.76 ab
Majestene 304	113.89 cd	839.67 bc	126.11 a	11.17 bc	47.11 b	3.00 bc
Majestene 305	105.44 cd	863.28 bc	124.44 a	11.39 bc	51.50 ab	3.00 bc
TerraClean 5.0	104.83 cd	864.22 bc	134.44 a	10.94 bc	50.33 ab	2.83 bc
Telone II Fumigated	229.80 ab	688.89 c	124.00 a	9.00 c	38.43 d	2.60 bc
***P*-values**	< 0.001	< 0.001	0.670	< 0.001	< 0.001	< 0.001

2.3.2 Greenhouse Experiments

In both years of the greenhouse trial evaluating the effect and damage potential of *M. hapla* on *Hemerocallis* spp. plants, daylily was an excellent host to *M. hapla* with RF values ranging from 1.82 to 11.98 in 2020 and from 199.71 to 1365.66 in 2021 (Table 2.3). *Meloidogyne hapla* final population densities ranged from 0 to 249,630 eggs/pot, including the control pots. As expected, the lowest inoculation rate of 500 *M. hapla* eggs/pot resulted in the lowest mean final population levels ($P \leq 0.021$). In 2020, the lowest inoculation rate also had the lowest gall ratings, and the highest inoculation rate had the highest gall rating index of 3.50 ($P < 0.001$). In 2021, the highest inoculum rate resulted in the highest final *M. hapla* population level and had the highest gall rating of 4.38 ($P < 0.001$). In both years, the control pots had a final population level of 0 *M. hapla* eggs/pot, a RF value of 0, and a gall rating of 0.

Table 2.3. Final mean *Meloidogyne hapla* population counts/pot, reproduction factor (RF) values, and gall rating based on five inoculation rates in the 2020 and 2021 greenhouse experiments. Values are the means of six replications. Means followed by the same letter within a column are not significantly different according to Tukey's honestly significant difference test ($P \leq 0.05$).

	2020			**2021**		
Inoculation Rate	**Final *M. hapla* eggs/pot**	**RF**[1]	**Gall Rating**[2]	**Final *M. hapla* eggs/pot**	**RF**	**Gall Rating**
0	0 b	0 b	0 c	0 c	0 b	0 d
500	5,990 b	11.98 c	0.38 c	85,353 bc	1,365.66 a	1.25 cd
3,000	26,085 a	8.70 bc	1.88 b	196,065 ab	522.84 a	2.25 bc
5,000	9,968 b	1.99 ab	1.75 b	168,685 ab	269.99 a	3.13 ab
10,000	18,190 ab	1.82 ab	3.50 a	249,630 a	199.71 a	4.38 a
***P*-values**	< 0.001	0.006	< 0.001	0.021	0.019	< 0.001

[1]RF (Reproduction Factor) values calculated as final nematode population density/initial nematode population density.

[2]Gall rating scores based on a scale of 0 to 5 where 0 = 0 galls, 1 = 1 to 2 galls, 2 = 3 to 10 galls, 3 = 11 to 30 galls, 4 = 31 to 100 galls, and 5 = > 100 galls per root system.

At the termination of the experiment in 2020, differences were observed among final plant measurements (Table 2.4). The control plants had the highest root weight, and the lowest inoculation rate resulted in the plants having the lowest shoot and root weights. Interestingly, the highest inoculation rate resulted in the highest shoot weights, the number of scapes, and the number of eyes/plant. Exploring the regression analysis (data not shown), the number of eyes, scapes, and growth index differed over time ($P \leq 0.001$), with the control plants having the highest growth rate and eyes, and the inoculation rate of 10,000 *M. hapla* eggs/pot having the highest number of scapes.

Table 2.4. Harvest fresh shoot and root weights (g), and year-long mean plant measurements based on the five *Meloidogyne hapla* inoculation rates in the 2020 and 2021 greenhouse trials. Values are the means of six replicates. Means followed by the same letter within a column are not significantly different according to Tukey's honestly significant difference test ($P \leq 0.05$).

	2020						2021					
Inoculation Rate	**Shoot Weight (g)**	**Root Weight (g)**	**Growth Index**[1]	**No. of Scapes/ Plant**	**No. of Buds/ Plant**	**No. of Eyes/ Plant**	**Shoot Weight (g)**	**Root Weight (g)**	**Growth Index**	**No. of Scapes/ Plant**	**No. of Buds/ Plant**	**No. of Eyes/ Plant**
0	131.38 a	382.75 a	37.95 ab	2.64 ab	2.38 a	7.67 b	59.38 a	295.38 a	36.20 c	1.94 b	1.78 a	7.97 b
500	121.75 a	277.13 a	38.68 b	2.29 a	1.25 a	6.95 a	55.25 a	298.38 a	35.84 c	1.62 ab	1.42 a	7.12 a
3,000	134.50 a	308.88 a	38.56 ab	2.75 b	1.75 a	7.87 b	59.88 a	294.63 a	33.65 b	1.39 ab	1.29 a	8.52 b
5,000	126.88 a	281.38 a	37.03 a	2.36 ab	1.88 a	6.69 a	41.25 a	232.63 b	31.03 a	1.11 a	0.86 a	7.02 a
10,000	144.88 a	345.38 a	38.67 b	3.41 c	2.25 a	7.94 b	48.63 a	204.38 b	32.61 ab	1.38 a	1.28 a	8.29 b
***P*-values**	0.493	0.335	0.013	< 0.001	0.907	< 0.001	0.082	< 0.001	< 0.001	< 0.001	0.666	< 0.001

[1]Growth Index (GI) values calculated as GI = (height + ((diameter 1 + diameter 2) / 2)) / 2.

In 2021, significant differences were observed in root weight ($P < 0.001$), growth index ($P < 0.001$), number of scapes ($P < 0.001$), and the number of eyes/plant ($P < 0.001$). The control plants had the second highest root weight, and the highest growth index and number of scapes, while the highest inoculation rate of 10,000 eggs resulted in the lowest root weight and the second lowest growth index. Over time, the regression analysis showed that the number of eyes, scapes, and growth index differed ($P \leq 0.001$), with the control plants resulting in the highest growth rate and the number of eyes and scapes.

2.4 DISCUSSION

This research investigated alternatives to fumigation to control plant-parasitic nematodes and found several viable options that provide promising *M. hapla* management leading to increased plant biomass and yield. All the alternative treatments tested here were applied every year in the spring, demonstrating that these new management strategies can be applied throughout the plant production cycle for long-term *M. hapla* management.

Looking at the soil and root *M. hapla* population levels, TerraClean 5.0 and Majestene 304 provided the best overall *M. hapla* management by 49% and 37%, respectively, compared to the control, with the Indemnify soil drench treatment providing promising control (a 21% *M. hapla* reduction compared to the control) and significantly increasing plant yields by 31%. In a similar study, the active ingredient of Majestene 304 (*Chromobacterium subtsugae*; Table 1) effectively decreased foliar plant-parasitic nematodes on several ornamental plants (Mitsuda, 2019). The effectiveness of fluopyram (the active ingredient in Indemnify) in controlling *Meloidogyne* spp. has been shown on a wide range of agricultural crops such as tomato (Dahlin et al., 2019), soybean (Ross, 2016), eggplant (Li et al., 2020), and lima bean (Jones et al., 2017), as an efficacious management option. In addition, fluopyram was shown to reduce *M. incognita* populations while increasing yield in squash (Nnamdi et al., 2022), which is similar to our findings. However, to our knowledge, this is the first study of fluopyram's effect on *M. hapla* in the floricultural industry, along with the products TerraClean 5.0 and Advanced Ag, since these products are still being registered for use in ornamentals.

Interestingly, Indemnify soil drench and root dip applications were not similar in their control of *M. hapla*, perhaps because the Indemnify root dip treatment was only applied to plants and not to the soil in the first year. This indicates that soil drench applications applied in the first year might be more successful in reducing *M. hapla* levels long-term instead of plant dips, which is the standard practice. Management may be more crucial in the first year since *M. hapla* can be present in the plant itself and therefore planted directly in the field (LaMondia, 1995; Wesemael et al., 2011). Even low initial populations of *M. hapla* have the potential to significantly increase on *Hemerocallis* spp. over three years (LaMondia, 1995).

Three different composts were tested with different manure bases. While composts can reduce *M. hapla* population levels in the soil compared to the untreated control, all three tested composts performed worse compared to the fumigated plot. In another study testing the same compost, Dairy Doo® with spelt, there was a significant reduction in lesion nematodes, *P. penetrans*, in a laboratory assay, but using higher quantities of compost than used in this study (Cole et al., 2020). Using differing rates of compost to manage *M. hapla* in ornamental field production should be further investigated.

Levels of *M. hapla* populations were low in the soil compared to inside the roots, particularly in 2019. In 2019, Michigan was substantially cooler and wetter than the other years in the field study, which may help explain this outcome. Other Michigan nematode research trials found similar results where soybean cyst nematode, *Heterodera glycines,* population levels also dropped in 2019 in a four-year soybean field trial (Thapa et al., 2022). The difference in population levels between the soil and roots could also be an indication that root examination of ornamentals may be more representative for sampling in long-term ornamental production fields. The natural nematode population spatial variability in the field, due to the patchiness of nematode distribution in the soil (Howland et al., 2014), may also have played a role in the lower soil population levels compared to inside the roots. Another factor may be the initial drop in *M. hapla* population levels after the application of the treatments in the spring of 2018; *M. hapla* population levels dropped on average 87% between spring and midseason in 2018 (the highest drop was with the Indemnify root dip treatment) and slowly increased throughout the rest of the three-year field cycle. However, even though the soil population levels were low, the population levels in the roots show treatment efficacy and combined, these results indicate that several of these treatments provide better nematode population reduction than fumigation.

When evaluating the effect of the treatments on plant growth, the Indemnify soil drench and Indemnify root dip treatments had the tallest plant heights, except in 2020 where TerraClean 5.0 had the highest, with the fumigated plants significantly having the smallest plant heights. The

Indemnify soil drench also had the highest final plant height, the largest root and shoot systems, the most eyes/plant, and the greatest yield, followed by the Indemnify root dip. This shows that Indemnify has a significant impact on increasing plant biomass and yield. Increased plant growth combined with more effective *M. hapla* management compared to the control and fumigated plants, make this management strategy very effective in ornamental plant production. Examining the fumigated final plant biomass data, the Telone II fumigated plants significantly had the lowest root weight, eyes/plant, plant heights, and yield, suggesting alternative management strategies can be more beneficial in the field production of daylily and that fumigation can significantly reduce plant biomass compared to even the control and other management strategies.

Several other plant-parasitic nematode species were found in the *Hemerocallis* spp. field trial, such as *Paratylenchus* spp., *Pratylenchus* spp., and *Paratrichodorus* spp. Similar studies also found *Pratylenchus* spp. and *Paratrichodorus* spp. in ornamental fields, but with only *Pratylenchus* spp. affecting ornamentals (Benson and Barker, 1985). *Paratylenchus shenzhenensis* can cause decline in ornamental anthuriums by infecting roots leading to decay (Wang et al., 2016). A study from Florida reported that *Paratylenchus* spp., *Pratylenchus* spp., and *Paratrichodorus* spp. were commonly found in daylily fields, but their effect on *Hemerocallis* spp. is unknown (Inserra et al., 1995). This is the first report of these plant-parasitic nematodes in ornamentals in Michigan.

The greenhouse study highlighted the importance of managing *M. hapla* in *Hemerocallis* spp. production. Even at the lowest inoculation rate, galls were present on daylily roots, which can lead to shipment rejection since daylily shipments are inspected before shipping. Due to this, finding new management options such as the ones tested in this field trial that reduces galls on plant roots is crucial. This is especially true since the *Hemerocallis* spp. plants had RF values above 1.0; thus, daylily is an excellent host for *M. hapla*. In a similar greenhouses study, *M. hapla* was also shown to successfully reproduce on *Hemerocallis* cv. ‘Bright Banner’ (LaMondia, 1996).

The significant plant damage observed at the differing *M. hapla* inoculation levels, especially in the second year of the greenhouse trial, indicates that there is likely economic damage occurring, but suggests that daylily can be tolerant of nematode feeding. Even though some of the final plant biomass measurements were not significantly different, the results of the regression analysis indicate that nematode infested plants can initially grow slower and have a reduction in desirable ornamental plant qualities, such as scapes and buds, compared to nematode-free plants. However, over the length of the daylily growth cycle, the plant can become more tolerant of its feeding and grow to similar sizes of nematode-free plants. It is important to note that these greenhouse findings may not always directly correlate to what is observed in the field due to the natural variability of nematodes in the soil and the tolerance of daylily to *M. hapla* feeding. However, the greenhouse trial clearly shows that even at low levels of *M. hapla* infection, the nematodes can readily feed and reproduce on daylily roots resulting in lower yields and shipment rejection.

2.5 CONCLUSION

In conclusion, we provide alternative nematode management strategies that can be applied throughout the production cycle in ornamental systems. We demonstrate that there are more effective solutions for *M. hapla* control in ornamental field production. Overall, TerraClean 5.0, Majestene 304, and Indemnify as a soil drench have the best potential for *M. hapla* management in daylily fields, with Indemnify outperforming Telone II fumigation by increasing yield by 40%. We also found that applying these treatments throughout the production cycle is practical and provides significantly better *M. hapla* management compared to current practices, such as pre-plant fumigation. The field results also accentuate that other plant-parasitic nematodes in ornamental field production may cause yield loss and therefore warrants further investigation. Lastly, through the greenhouse trial, these results indicate that even low population levels of *M. hapla* can affect plant growth and can negatively impact plant sales due to the presence of their galls on the roots and highlight the importance of *M. hapla* management. This study therefore fills a knowledge gap on these crucial pests in ornamentals and deliver practical outcomes for the floriculture industry.

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CHAPTER 3: NEW MANAGEMENT SYSTEMS TO CONTROL THE NORTHERN ROOT-KNOT NEMATODE (*MELOIDOGYNE HAPLA*) IN DAYLILY (*HEMEROCALLIS* SPP.) PRODUCTION FIELDS WITH HOST STATUS TRIALS TO *PARATYLENCHUS* SPP.

3.1 INTRODUCTION

Daylily (*Hemerocallis* spp.) is one of the most economically important ornamental plants in the United States (Mosonyi et al., 2019; Gatlin, 1999) with an economic value over $16.8 million in 2020 (USDA, 2021). Daylilies are a perennial, herbaceous monocot and are widely cultivated, with approximately 20 species and over 98,000 registered cultivars in the United States (American Daylily Society, 2023; Gulia et al., 2009). These ornamental plants have a wide array of uses, such as for food, medicine, beautification, and environmental conservation such as preventing soil erosion (Wali et al., 2022; Munson, 1989). In the United States, daylilies are a major component of the Michigan ornamental plant industry; Michigan has the third largest floriculture industry in the United States.

Daylilies are asexually propagated and are grown in the field for three years. After three years, they are harvested and separated into smaller plants, called eyes, by dividing its crown; each eye can be sold as a new daylily plant. Since these plants are grown in the field for several years, daylilies can be plagued by a number of pathogens. However, the most important pathogen group afflicting daylily and other perennial plant production fields in the northern North America are plant-parasitic nematodes, specifically the northern root-knot nematode, *Meloidogyne hapla*, which can cause over 20% yield loss in daylily production (Howland et al., 2022; Lindberg et al., 2018; LaMondia, 1996).

Plant-parasitic nematodes are a global pathogen, and the most economically important plant-parasitic nematode is the root-knot nematode, *Meloidogyne* spp., due to its large host range of over 3,000 plant species (Abad et al., 2003) and widespread distribution. Root-knot nematodes are sedentary endoparasites and establish permanent feeding sites called giant cells in susceptible host roots. Inside the roots, root-knot nematodes molt three times (second-stage juvenile (J2) to J4) until they become an adult, and their growth causes the formation of galls on the roots (Taylor and Sasser, 1978). These characteristic galls on the roots can prevent the sale of some plants for export, such as daylilies (Howland et al., 2022). Small galls with usually a single nematode occur on young feeder roots and larger galls can be a consequence of multiple infections at the same location. Female root-knot nematodes lay eggs in a gelatinous matrix called an egg mass outside the roots; a single egg mass can contain 400-500 eggs. Under optimal conditions, the lifecycle of this nematode can take four to five weeks to complete, producing four to six generations per season (Hussey and Janssen, 2002; de Guiran and Ritter, 1979; Williams, 1974).

Another plant-parasitic nematode commonly found in Michigan ornamental plant fields are pin nematodes, *Paratylenchus* spp. (Howland et al., 2022). Unlike root-knot nematodes, pin nematodes are migratory ectoparasites that feed on the exterior surfaces of host roots. These nematodes are unique in the fact that before their final molt to an adult, they develop into pre-adults (J4 stage), where the nematode lacks a complete stylet and does not feed. In this stage, pre-adult pin nematodes can survive for long periods of time in unfavorable conditions such as temperature and moisture extremes, and therefore can persist in soils for years (Rhoades and Linford, 1959). However, if environmental conditions are favorable, the J4 nematode will not stay in this resting stage and will molt into an adult. Female pin nematodes can lay one to three eggs per day and can complete a generation in 36 to 38 days under optimal conditions (Wood, 1973).

Pin nematodes feed on plant roots for the majority of its developmental stages (Loof, 1975; Wood, 1973; Eck, 1970), yet the damage caused by *Paratylenchus* spp. on daylily plants is unknown (Inserra et al., 1998), along with any management strategies. Also unknown is how good a host *Hemerocallis* spp. is to pin nematodes. Howland et al. (2022) found high population levels of pin nematodes in daylily fields; therefore, establishing a threshold value for pin nematode is very important. Determining this baseline threshold level will save both time and money: if *Hemerocallis* spp. can withstand low or moderate pin nematode population levels without an impact on plant yield then growers may not need to fumigate or apply chemicals every year to control them. This information can also help determine how much focus future research efforts need to be geared to nematodes other than *Meloidogyne* spp. on ornamental plants.

Nematodes are extraordinarily difficult to manage and almost impossible to eradicate since they can remain in the soil for many years even without a host. Current management strategies for *M. hapla* in ornamental field production are limited to two main options: hot water dips and preplant fumigation. Hot water dips are where daylily rootstocks are ‘dipped’ in 41.7°C hot water for one hour and then they are cooled down to 12.8°C for thirty minutes. While this method can kill plant-parasitic nematodes in the plant before it is planted in the field, it can also cause up to 50% mortality of the propagules and can reduce vigor on some varieties, further escalating the detrimental impact of these nematodes on daylilies (Poley et al., 2018; Daughtrey and Benson, 2005; Inserra et al., 1995).

Preplant fumigation is where nematicides are injected into the soil and then covered with a polyethylene tarp or mulch. For decades, soil fumigation has been a main tactic to control plant-parasitic nematodes in agricultural systems throughout the United States (Zasada et al., 2010). In most crops such as vegetable crops, preplant fumigation is the dominant management strategy for root-knot nematodes (Hajihassani, 2018). Preplant soil fumigation is very effective in annual production systems, but in ornamental plant fields that are in production for several years, fumigation only controls nematodes in the first year and provides just 60-70% control of root-knot nematodes in Michigan ornamental production fields. Additionally, preplant soil fumigation does not effectively manage pin nematode population levels (Howland et al., 2022). This drastically emphasizes the need for better management options of plant-parasitic nematodes in ornamental production that also focuses on keeping nematode population levels low throughout the growing season.

A recent study to determine alternative management strategies beyond hot water dips and preplant fumigation showed several promising management options that can be applied throughout the daylily production cycle (Howland et al., 2022). One of the more attractive options was Indemnify nematicide (ai: fluopyram, Bayer Environmental Science) as plant dip since it can be easily implemented in the ornamental plant industry since they already have the plant dipping infrastructure in place. Other promising strategies were TerraClean 5.0 (ai: hydrogen peroxide, BioSafe Systems), Majestene 304 (ai: *Chromobacterium subtsugae*, Marrone Bio Innovations), AzaGuard (ai: neem oil, BioSafe Systems), and Indemnify as a soil drench. However, as these treatments still allowed *M. hapla* reproduction on daylily roots, which can prevent plant exports or cause fields to become quarantined since ornamental plants are inspected before harvest and exportation, new field trials to test a combination of treatments, such as Indemnify as a root dip plus other treatments, and the best compost from the Howland et al. (2022) field study which helped increase plant growth in combination with other treatments needs to be evaluated.

Therefore, two field trials were conducted to test 1) Indemnify as a root dip + other treatments, and 2) a high carbon compost + other treatments on their effect on *M. hapla* and *Paratylenchus* spp. population levels. Another objective will be to take the best treatments from the field trials and conduct a greenhouse experiment to confirm the results and see if these management options can be applied in the greenhouse, where nematodes are also found. The last objective will be to conduct a greenhouse trial to test the host status of *Paratylenchus* spp. on daylily in comparison to a known host and to determine their impact on daylily plants. The results of these studies will be to determine new management systems to manage *M. hapla* effectively and sustainably in ornamental plant fields, reduce the number of galls on plant roots, and provide knowledge on how much research and management focus needs to be concentrated on *Paratylenchus* spp. in the Michigan ornamental plant industry.

3.2 METHODOLOGIES

3.2.1 Indemnify Dip Field Trial

At a commercial ornamental nursery in Zeeland, MI, a replicated three-year field trial was conducted to test how effective multiple combinations of new management strategies are in controlling *Meloidogyne hapla* and *Paratylenchus* spp. population levels in the field. The field trial was established from 2019 to 2021 in a field with a known *Paratylenchus* spp. history and a low population level of *M. hapla.* The soil type was Chelsea loamy fine sand (Web Soil Survey, 2020).

In conjunction with dipping the plants in Indemnify as a preplant dip, five other treatments were chosen based on the results of the field trial conducted by Howland et al. (2022). The treatments selected were 1) Indemnify Dip + Indemnify Drench, 2) Indemnify Dip + TerraClean 5.0, 3) Indemnify Dip + AzaGuard, 4) Indemnify Dip + 101 Starter Blend Compost, 5) 101 Starter Blend Compost Only (by itself), and 6) an untreated control (Table 3.1). The 101 Starter Blend Compost Only treatment was chosen for economic reasons since it was the most cost-effective treatment and is widely available for purchase. Additionally, this nursery uses this potting compost in all its greenhouse pots, and we wanted to see if it contained any nematicidal properties.

Table 3.1. Characteristics and application rates of the treatments applied in the *Hemerocallis* spp. dip field trial from 2019-2021 to manage *Meloidogyne hapla* during a three-year daylily production cycle in a commercial ornamental nursery in Zeeland, Michigan.

Treatments	Manufacturer	Active Ingredient	Rate	2019	2020	2021
Indemnify Root Dip + Indemnify Drench	Bayer Environmental Science	Fluopyram	15 ml/15.14 L Dip; 41.11 L/ha Drench	Indemnify Dip + Drench	Indemnify Drench	Indemnify Drench
Indemnify Root Dip + 101 Starter Blend Compost	Morgan Composting Inc.	High carbon potting soil	15 ml/15.14 L Dip; 1001.53 kg/ha Compost	Indemnify Dip + Compost Raked in Topsoil	Compost Raked in Topsoil	Compost Raked in Topsoil
Indemnify Root Dip + AzaGuard	BioSafe Systems	Neem oil	15 ml/15.14 L Dip; 0.27 L/ha AzaGuard	Indemnify Dip + AzaGuard Drench	AzaGuard Drench	AzaGuard Drench
Indemnify Root Dip + TerraClean 5.0	BioSafe Systems	Hydrogen peroxide	15 ml/15.14 L Dip; TerraClean 5.0: Full: 41.56 L/ha Half: 20.78 L/ha	Indemnify Dip + TerraClean Drench – Full Rate	TerraClean Drench – Half Rate	TerraClean Drench – Half Rate
101 Starter Blend Compost	Morgan Composting Inc.	High carbon potting soil	1001.53 kg/ha Compost	Indemnify Dip + Compost Raked in Topsoil	Compost Raked in Topsoil	Compost Raked in Topsoil
Untreated Control	--	Untreated Control	--	No Treatment	No Treatment	No Treatment

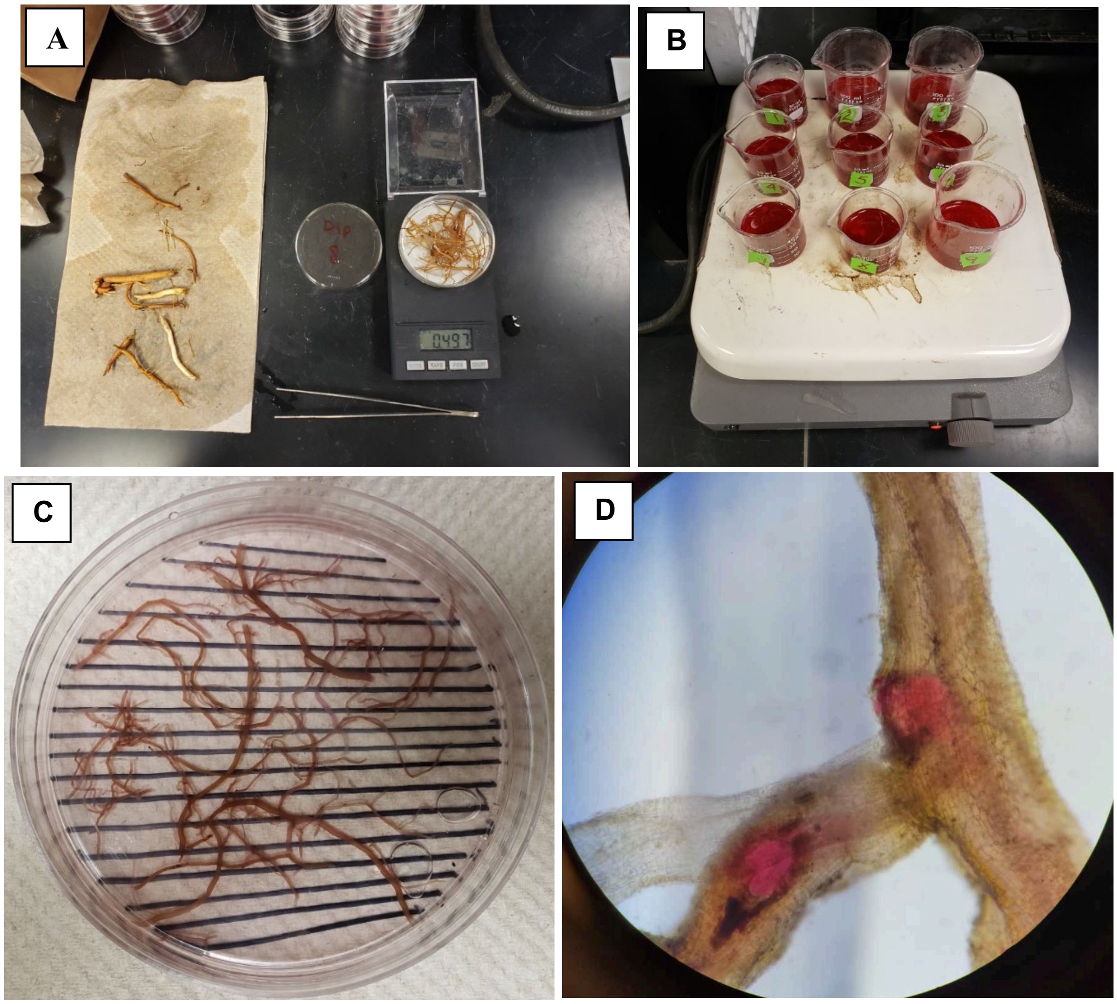
The field was divided into 30 plots, six treatments with five replicates. Treatments were assigned to each plot using Agriculture Research Manager software (Gylling Data Management Inc., Brookings, SD). Each plot was 2.5 m x 1.2 m with the daylily plants planted 0.18 m apart. Prior to planting in the spring of 2019, the daylily plant cv. 'Orange Smoothie,' was first submerged in a 15 ml/15.14 L Indemnify solution for 8 minutes for all treatments except for the untreated control and the 101 Starter Blend Compost Only treatment. The Indemnify drench treatments, TerraClean 5.0 treatments, and AzaGuard treatments were hand poured onto each plot using 11.4 L watering cans and poured in furrow in the planting row of each respective plot. The compost was raked into the topsoil. The daylily plants were hand planted into each respective plot according to industry standards. Treatments were applied each year in the spring at the same rate as the first application in 2019, except for TerraClean 5.0 since it is phytotoxic; it was applied at a half rate in 2020 and 2021.

At the time of the treatment applications in the spring of 2019, each plot was soil sampled. Soil samples were composite samples (multiple samples taken and homogenized) taken randomly throughout each respective plot using a soil probe, 25.4 cm by 2.54 cm. Ten soil samples were taken in the root zone in a zigzag pattern in each plot, placed in labeled plastic bags, and kept at 10°C until processing. Soil samples were also taken at midseason in July and in the fall in October. Soil samples were taken three times/year for the duration of the trial.

Soil samples were processed according to standard sucrose centrifugal-flotation methods (Ingham, 1994; Barker et al., 1969; Jenkins, 1964) to extract plant-parasitic nematodes from each soil sample. Briefly, the soil in each bag was thoroughly shaken and a 100 cm^3 subsample was removed to be processed. The soil sample was mixed with water and then poured over nested 250-µm and 25-µm sieves; the nematodes were retained on the 25-µm sieve. After repeating this process three times, the contents of the 25-µm sieve were centrifuged with a 40% sucrose solution. Centrifuging the sample with the sucrose solution will cause the nematodes to be suspended in the solution and the soil particles to fall to the bottom of the container purifying the sample from debris and soil. Each sample was then poured over a 25-µm sieve and rinsed for 30 seconds to remove the sucrose from the nematodes. The final contents of the 25-µm sieve were enumerated on an inverted microscope; all plant-parasitic nematodes were counted (Howland et al., 2022).

At the midseason sampling period, root samples and plant height measurements were taken as well. Plant height was taken using a measuring stick to record the heights of three plants and then they were averaged together. Root samples were taken using a shovel to gather a subsample of fine roots from three plants/plot. Root samples were processed and stained with acid fuchsin stain according to protocol (Byrd et al., 1983). Briefly, 1 g of each root sample was gently washed free of soil (Figure 3.1A), placed in a 10% NaOCl solution for 4 minutes, rinsed under running water, and then soaked in tap water for 15 min. The roots were then drained of all water and placed in a 50 ml glass beaker with 1 ml acid fuchsin stain and 30 ml tap water. Each beaker was boiled for 1 minute on a hot plate (Figure 3.1B), let cool to room temperature, and then drained of the acid fuchsin stain solution. The roots were then placed back into the beakers and 30 ml of acidified glycerin destaining solution was added to each beaker. The beakers were then heated until boiling, cooled to room temperature, and placed in a gridded petri dish for counting (Figure 3.1C). The acid fuchsin stains the nematodes inside the roots a bright pink to facilitate counting (Figure 3.1D). All life stages of *M. hapla* were counted.

Figure 3.1. Processing root samples for staining endoparasitic nematodes. (**A**) Weighing out 1 g of root to be processed. (**B**) Boiling the root samples in acid fuchsin stain. (**C**) A root sample in a gridded petri dish for counting. (**D**) Two adult *Meloidogyne hapla* stained pink inside a daylily root.



In October 2021, this field trial ended. Final soil samples were collected and final plant height measurements were taken. Additional plant measurements, such as shoot and root fresh weights (g), crown width (cm), the number of eyes/plant, and yield, were taken from three plants that were dug up in each respective plot. Yield was calculated by determining the number of industry standard ratings of G1 (Grade 1) propagules for each individual plant.

3.2.2 High Carbon Compost Field Trial

A three-year field trial was conducted from 2020 to 2022 at a commercial ornamental plant nursery in Zeeland, MI, to test the best combinations of the previous two field trials in combination with a compost blend that nursery now uses in all its fields due to its plant growth benefits and from the results of Howland et al. (2022). Prior to beginning the field trial, fields were scouted to locate areas with a high *M. hapla* population level; the field with the highest population level was used in this study. The same soil type present in the other two field trials, Chelsea loamy fine sand, was the soil in this field (Web Soil Survey, 2020).

Seven treatments were selected based on the results of the Dip Field Trial (above) and the first field trial at this nursery (Howland et al., 2022) (Table 3.2). To see how effective dipping the plants in Indemnify was compared to the soil drench applications of Indemnify, three treatments were chosen to differentiate them: dip + drench, dip only, and drench only. The prescriptive blend compost used in this trial is the same one used in the first field trial (Compost 2) from Howland et al. (2022) but applied at a higher rate used by the nursery. There were two controls in this trial: an untreated control of no compost nor chemicals applied, and a control of only compost to determine how effective the compost is as a management option by itself; this compost is different than the one used in the Dip Field Trial. The other treatments were selected due to their effective management of *M. hapla* from the results of the previous two field trials. Therefore, the treatments were: 1) Compost + Indemnify Dip + Indemnify Drench, 2) Compost + Indemnify Dip, 3) Compost + Indemnify Drench, 4) Compost + Indemnify Dip + TerraClean 5.0, 5) Compost + Indemnify Dip + AzaGuard, 6) Compost (by itself), and 7) an untreated control (Table 3.2).

Table 3.2. Characteristics and application rates of the treatments applied in the *Hemerocallis* spp. compost field trial from 2020-2022 to manage *Meloidogyne hapla* during a three-year daylily experiment at a commercial ornamental nursery in Zeeland, Michigan.

Treatments	**Manufacturer**	**Active Ingredient**	**Rate**	**2020**	**2021**	**2022**
Compost	Morgan Composting Inc.	Composted dairy cow manure with wood ash	1001.53 kg/ha Compost	Compost Raked in Topsoil	Compost Raked in Topsoil	Compost Raked in Topsoil
Compost + Indemnify Dip + Indemnify Drench	Bayer Environmental Science	Fluopyram	15 ml/15.14 L Dip; 41.11 L/ha Drench	Indemnify Dip + Drench+ Compost Raked in Topsoil	Drench; Compost Raked in Topsoil	Drench; Compost Raked in Topsoil
Compost + Indemnify Dip	Bayer Environmental Science	Fluopyram	15 ml/15.14 L Dip	Indemnify Dip + Compost Raked in Topsoil	Compost Raked in Topsoil	Compost Raked in Topsoil
Compost + Indemnify Drench	Bayer Environmental Science	Fluopyram	41.11 L/ha Drench	Drench + Compost Raked in Topsoil	Drench; Compost Raked in Topsoil	Drench; Compost Raked in Topsoil
Compost + Indemnify Dip + AzaGuard	BioSafe Systems	Neem oil	15 ml/15.14 L Dip; 0.27 L/ha AzaGuard	Indemnify Dip + Drench + Compost Raked in Topsoil	Drench; Compost Raked in Topsoil	Drench; Compost Raked in Topsoil

Table 3.2. (cont'd)

Compost + Indemnify Dip + TerraClean 5.0	BioSafe Systems	Hydrogen peroxide	15 ml/15.14 L Dip; TerraClean 5.0: Full: 41.56 L/ha Half: 20.78 L/ha	Indemnify Dip + Drench – Full Rate; Compost Raked in Topsoil	Drench – Half Rate; Compost Raked in Topsoil	Drench – Half Rate; Compost Raked in Topsoil
Untreated Control (Nothing)	--	Untreated Control	--	No Treatment	No Treatment	No Treatment

The compost was first raked into the soil of each respective plot, and then the daylily plants, cv. 'Orange Smoothie' were hand-planted into the field after being dipped in Indemnify for 8 minutes depending on the treatment. Drench applications were applied using 11.4 L watering cans and poured in furrow in the planting row of each respective plot. Treatments were applied in the spring all three years of this trial. All treatments were applied at the same rate each year except for TerraClean 5.0 since it is phototoxic; therefore, TerraClean 5.0 was applied at a half rate to each respective plot in years two and three. Each treatment had five replicates and were arranged in a randomized block design in the field. Treatments were assigned to each plot using Agriculture Research Manager (ARM) software. Therefore, the field was divided into 35 plots (7 treatments with 5 replications). Each plot consisted of 36 plants and was arranged 4 rows wide by 9 rows deep with a plot size of 2.8 m by 1.8 m.

Similar to the Dip Field Trial, soil samples were taken three times a year: in the spring in May, at the midseason in August, and in the fall in October. Soil samples were composite samples; ten samples were taken in a zigzag pattern and then placed in labelled bags and kept at 10°C until processing. Soil samples were processed according to standard sucrose centrifugal-flotation methods (Ingham, 1994; Barker et al., 1969; Jenkins, 1964). At the midseason soil sampling, root samples, number of eyes/plant, and plant height measurements were taken from each plot. Root samples were taken as described in the Dip Field Trial: roots were dug up with a shovel, placed in labelled bags, and kept at 10°C until processing. One gram of each root sample was then processed according to protocol (Byrd et al., 1983) and the number of *M. hapla* inside each root system was enumerated on an inverted microscope. Plant height was taken by using a measuring stick to record the heights of three plants and then they were averaged together; the same three plants that had their heights measured, the number of eyes/plant were also counted.

At the end of this trial in October 2022, final soil samples were collected as described above and final plant height and eye measurements were taken. Additionally, three plants from each plot were dug up to take further measurements from such as shoot and root fresh weight (g), crown width (cm), and yield. Yield was calculated by determining the number of industry standard ratings of G1 offshoots for each individual plant.

3.2.3 Alternative Methods Greenhouse Trial

In conjunction with the field trials to determine new, alternative methods to control *M. hapla* in daylily plants, a two-year greenhouse trial was conducted at the Michigan State University's Plant Greenhouses, East Lansing, MI. A two-year greenhouse trial was implemented since daylilies can also be grown in the field for just two years, not always three years. Therefore, the trial was conducted from May 2021 until September 2022. Nursery-grade bare-rooted *Hemerocallis* spp. cv. 'Going Bananas' plants were obtained in the late spring of 2021 and each individual daylily plant was potted in a 1:1 mix of steam-pasteurized sandy loam soil:sand in 3.7 L pots.

The newly potted *Hemerocallis* plants were left to grow for two weeks and then inoculated with 9,000 *M. hapla* eggs/pot. *Meloidogyne hapla* collected from a daylily field in Zeeland, MI was used as inoculum; the nematodes were reared on tomatoes (*Lycopersicon esculentum* Mill. cv. 'Rutgers;' Burpee Seeds, Warminster, PA) to maintain their colony. Inoculum was obtained by destructively harvesting the tomato plants and collecting eggs from washed roots by agitating the root system in a 0.01% NaOCl solution for four minutes (Hussey and Barker, 1973). Plants were inoculated by aliquoting the inoculum into four, 5-cm deep holes in the soil around the base of the plant and then covered up with clean sand. After two weeks to allow the nematodes to become established, respective treatments were applied to each pot according to label rates (Table 3.3). There were 6 treatments: 1) AzaGuard, 2) A High Carbon Compost (from the Compost Field Trial), 3) Indemnify, 4) Majestene 304, 5) TerraClean 5.0, and 6) an untreated control. Treatments were applied in the spring of both years of the trial to mimic how the plants are managed in the field. Each treatment was replicated six times and the plants were arranged in a randomized block design in the greenhouse.

Table 3.3. Characteristics and application rates of the treatments applied in a two-year *Hemerocallis* spp. greenhouse trial to find alternative methods to manage *Meloidogyne hapla*.

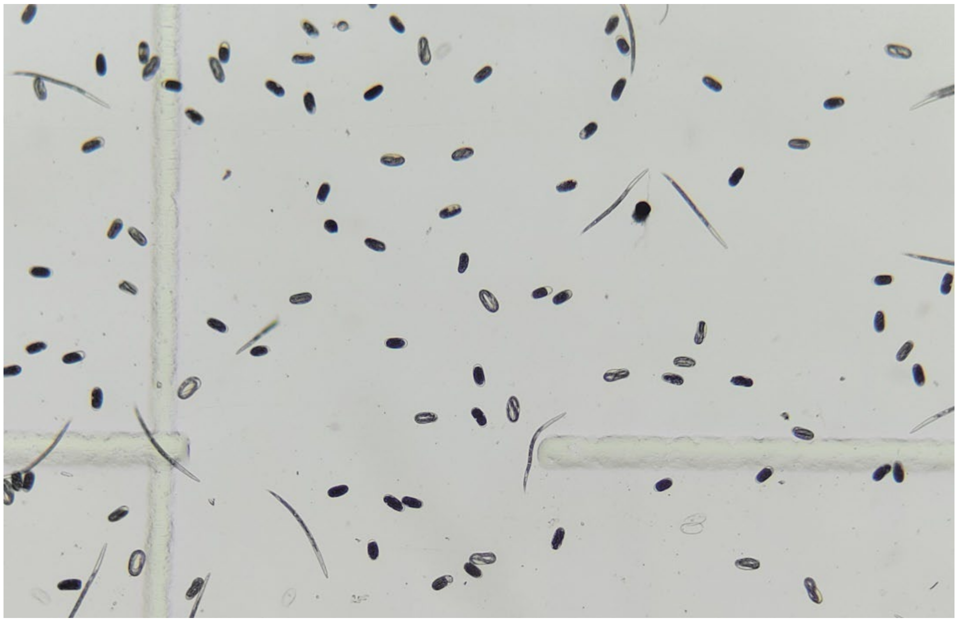
Treatments	Manufacturer	Active Ingredient	Rate	Spring 2021	Spring 2022
AzaGuard	BioSafe Systems	Neem oil	0.023 ml/pot	Drench	Drench
Compost	Morgan Composting Inc.	Composted dairy cow manure with wood ash	25.79 g/pot	Mixed into Soil	Mixed into Soil
Indemnify	Bayer Environmental Science	Fluopyram	0.0042 ml/pot	Drench	Drench
Majestene 304	Marrone Bio Innovations	*Chromobacterium subtsugae*	18.14 g/pot	Drench	Drench
TerraClean 5.0	BioSafe Systems	Hydrogen peroxide	0.63 ml/pot	Drench	Drench
Untreated Control	--	Untreated Control	--	No Treatment	No Treatment

The plants were fertilized biweekly (15 ml/7.6 L, Peters' Professional 20-10-20 N-P-K, ICL Specialty Fertilizers, Dublin, OH), had a 16h:8h light:dark photoperiod, and were kept at 26°C. Plant quality parameters, such as plant height (cm), number of eyes, number of flower buds, and number of scapes were taken biweekly. Additionally, two width diameters (cm) of the plant, N-S and E-W, were measured to generate a growth index (Krug et al., 2010).

At the end of the experiment in September 2022, final plant measurements were taken, such as plant height and diameter measurements (cm), number of eyes, number of flower buds, number of scapes, and crown width (cm). Plants were then destructively harvested by cutting off the foliage and removing the soil from the roots to obtain shoot and root fresh weights (g). A *M. hapla* gall rating was taken on a scale of 0 to 5 where 0 = 0 galls, 1 = 1 to 2 galls, 2 = 3 to 10 galls, 3 = 11 to 30 galls, 4 = 31 to 100 galls, and 5 = > 100 galls per root system (Taylor and Sasser, 1978). Lastly, final *M. hapla* population levels were obtained through extracting eggs from each individual root system according to standard protocols (Byrd et al., 1972; Hussey and

Barker, 1973; Jenkins, 1964) and enumerating the eggs under an inverted microscope (Figure 3.2).

Figure 3.2. Light micrograph of *Meloidogyne hapla* eggs and second-stage juveniles extracted from *Hemerocallis* spp. plant roots in a gridded counting dish.



3.2.4 *Paratylenchus* spp. Greenhouse Trial

A replicated greenhouse trial was conducted to determine the host status and impact of *Paratylenchus* spp. on daylily plants in the Applied Nematology Lab's Plant Greenhouse at Michigan State University in East Lansing, MI. There were four treatments in this trial with the aim to test the host status of *Paratylenchus* spp. on daylily plants in comparison against a known host, sweet corn (*Zea mays*) (Siddiqui et al., 1973). The second aim of this trial was to test the impact of *Paratylenchus* spp. feeding on daylily plants; therefore, one of the treatments was uninoculated daylily plants. A treatment of plain soil was included to see if *Paratylenchus* spp. just survived in the soil as pre-adult J4s or actually reproduced on the plant in each pot. Therefore, the treatments were: 1) Corn (positive control), 2) Plain Soil (negative control), 3) Daylily- inoculated, and 4) Daylily- non-inoculated.

Similar to the experiment described above in the Alternative Methods Greenhouse Trial, nursery-grade bare-rooted *Hemerocallis* spp. cv. 'Going Bananas' were potted into a 1:1 mix of pasteurized greenhouse soil and pure sand in 3.7 L pots. Two sweet corn seeds/pot (*Zea mays* cv. 'Triple Crown White;' Burpee Seeds, Warminster, PA) were planted in the corn treatment pots in the same soil mixture and then thinned to one corn plant/pot. After two weeks, respective pots were inoculated with 1,000 mixed-stage individuals/pot of *Paratylenchus* spp. in the root zone. *Paratylenchus* spp. originally collected from a daylily field in Zeeland, MI, and reared on tomato (*Lycopersicon esculentum* Mill. cv. 'Rutgers;' Burpee Seeds, Warminster, PA) was used as inoculum. Plants were then arranged in a randomized block design with five replications.

Plants were kept at a 16h:8h light:dark photoperiod at 26°C and fertilized biweekly (15 ml/7.6 L, Peters' Professional 20-10-20 N-P-K, ICL Specialty Fertilizers, Dublin, OH). Plant height (cm), number of eyes, number of flower buds, number of scapes, and two width diameters (cm) N-S and E-W were taken biweekly on the daylily plants throughout the duration of this experiment to determine any effect on daylily plants from *Paratylenchus* spp. The plant height and two width diameters were used to generate a growth index (Krug et al., 2010).

After five months, the plants were destructively harvested. Final plant measurements were taken only from the daylily plants since the aim of this trial was to determine the impact of *Paratylenchus* spp. on daylily, and the corn plants were only included as a positive control. Therefore, plant shoot and root fresh weights (g) and crown width (cm) were recorded for the two daylily treatments. Final plant height, and the number of eyes, flower buds, and scapes were also recorded. The soil from each pot was placed in labelled Ziploc bags (SC Johnson, San Diego, CA) to extract *Paratylenchus* spp. from; the soil was kept at 10°C until processing. The final population of *Paratylenchus* spp. was determined by extracting nematodes from a 100 cm^3 subsample of soil according to standard sucrose centrifugal-flotation methods as described above in the field trials (Ingham, 1994; Barker et al., 1969; Jenkins, 1964). Nematodes collected from the soil were enumerated on an inverted microscope and a reproduction factor (RF), RF= final nematode population/initial nematode population (Oostenbrink, 1966), was calculated. A RF value > 1 indicates that the plant is a good host while a RF value < 1 indicates a poor host; a RF value of 0 indicates the plant is a non-host. This greenhouse trial was repeated once and both trials were conducted concurrently.

3.2.5 Statistical Analyses

Data from the field and greenhouse trials were analyzed in R 4.0.3 (R Core Team, 2020). Data distribution was assessed before analysis and was log10(x + 1) transformed to meet normality assumptions, if needed. Analysis of variance (ANOVA) was used followed by means separation to determine if there was a significant difference in each respective experiment among the treatments for *M. hapla* data, *Paratylenchus* spp. data, and plant measurement data. Additionally, a repeated measures analysis using linear regression was also conducted on the biweekly plant evaluations taken throughout the duration of the greenhouse trials, such as the growth index measurements, number of eyes, number of flower buds, and number of scapes. Tukey's honest significance difference test ($P \leq 0.05$) was used to determine differences among treatments in the 'emmeans' package in R (Lenth, 2019).

3.3 RESULTS

3.3.1 Indemnify Dip Field Trial

Meloidogyne hapla population levels varied by treatment throughout the duration of the field trial ($P < 0.001$). Population levels ranged from 0 to 72 *M. hapla*/100 cm^3 soil during the trial (Figure 3.3). Similarly, *Paratylenchus* spp. population levels varied by treatment throughout the three years of the trial ($P < 0.001$). *Paratylenchus* spp. population levels ranged from 0 to 4,464 *Paratylenchus* spp./100 cm^3 soil (Figure 3.4). For both nematodes, the highest population levels occurred in the third year. Across all three years, the control plots had the lowest *M. hapla* population levels with an average of 0.60 *M. hapla*/100 cm^3 soil, while the 101 Starter Compost Only treatment had significantly higher population levels with an average of 7.60 *M. hapla*/100 cm^3 soil. For the *Paratylenchus* spp., across all three years, the Indemnify Dip + 101 Starter Compost treatment had the lowest population levels of 46.67 *Paratylenchus* spp./100 cm^3 soil, while the control plots had the highest of 807.93 *Paratylenchus* spp./100 cm^3 soil.

Figure 3.3. Mean ± SEM *Meloidogyne hapla*/100 cm^3 of soil for each treatment (N=5) in the dip field trial in 2019, 2020, and 2021.

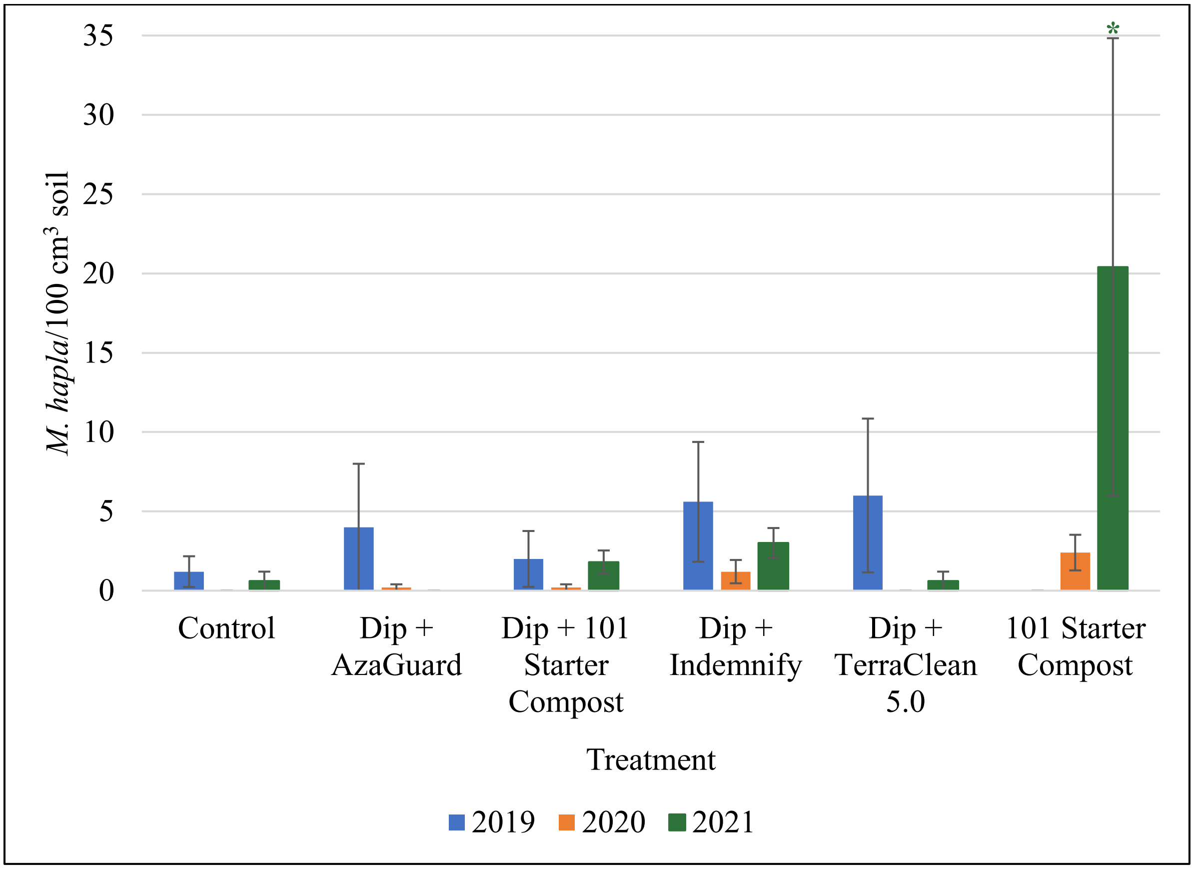
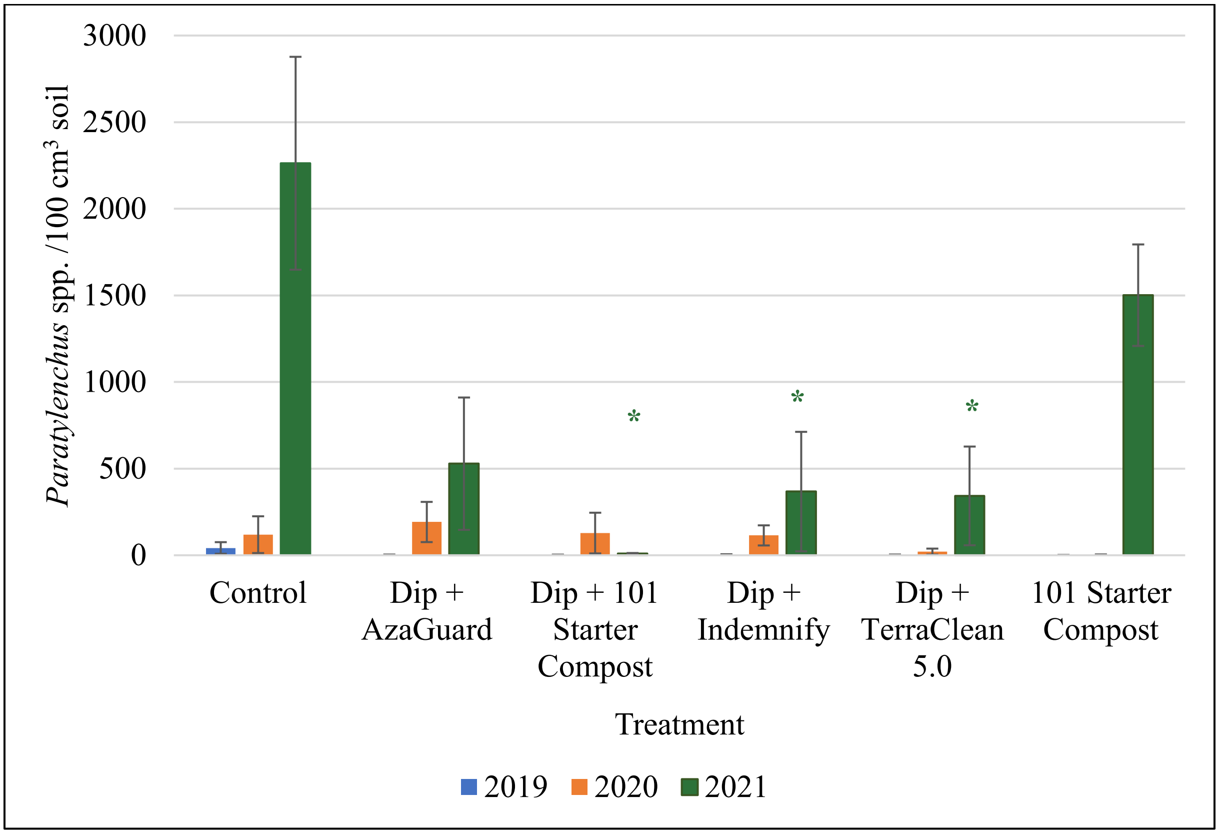
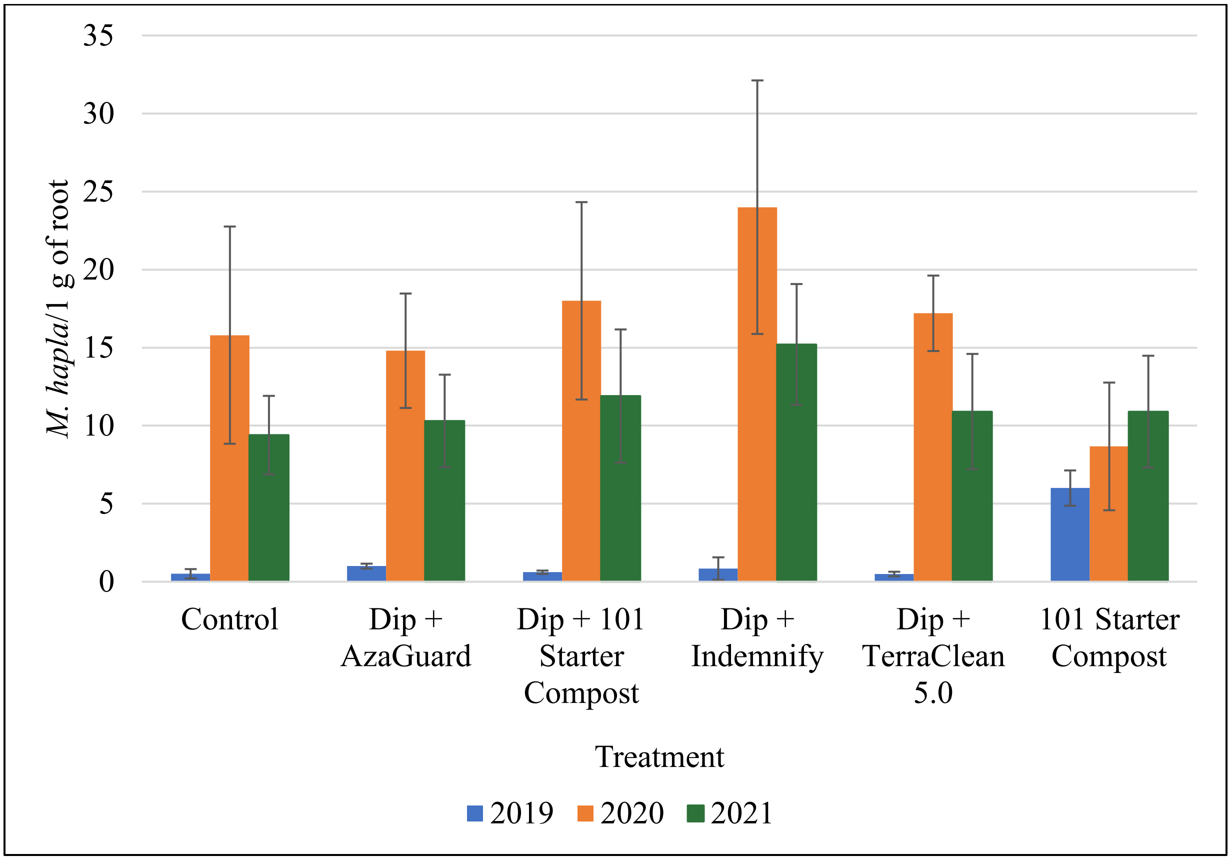


Figure 3.4. Mean ± SEM *Paratylenchus* spp./100 cm^3 of soil for each treatment (N=5) in the dip field trial in 2019, 2020, and 2021.



Examining the root analysis of *M. hapla*/1 g of *Hemerocallis* spp. root, population levels also varied by treatment over time throughout the duration of the trial ($P < 0.001$). Both the control plots and the 101 Starter Compost Only treatment had the lowest overall population levels of *M. hapla* inside the roots, of 8.52 and 8.57 *M. hapla*/1 g of root, respectively, while the Indemnify Dip + Indemnify Drench treatment had the highest overall population level of 13.35 *M. hapla*/1 g of root. Similarly, the midseason plant height data (data not shown) varied by treatment throughout the duration of the trial ($P < 0.001$), with the 101 Starter Compost Only treatment having the lowest overall plant heights of 37.79 cm and the Indemnify Dip + Indemnify Drench treatment had the overall tallest plant heights of 49.10 cm.

Figure 3.5. Mean ± SEM midseason *Meloidogyne hapla*/1 g of *Hemerocallis* spp. root for each treatment (N=5) in the dip field trial at midseason in July in 2019, 2020, and 2021.



When the experiment was terminated in October 2021, shoot and root fresh weights (g), the number of eyes/plant, crown width (cm), and yield (G1) were taken from three plants/plot (Table 3.4). All the data was not significant except for shoot weight (g). The control plants significantly had the smallest shoot weights of 165.07 g and the Indemnify Dip + TerraClean 5.0 had the largest of 220.40 g. Identically, the control plants had the smallest root system weight of 1302.87 g and the Indemnify Dip + TerraClean 5.0 plants had the largest root weight of 1808.07 g. Looking at the number of eyes/plant, again the control plants had the least amount of eyes at 11.80 eyes/plant while the Indemnify Dip + TerraClean 5.0 plants had the most number of eyes/plant of 15.47. The Indemnify Dip + Indemnify Drench treatment had the smallest average crown width of 15.40 cm, while the 101 Starter Compost Only treatment had the largest crown width of 18.80 cm. Lastly, looking at yield, the Indemnify Dip + Indemnify Drench plants had the highest yield of 2.80 G1s and the 101 Starter Compost Only treatment plants had the lowest yield of 1.80 G1s.

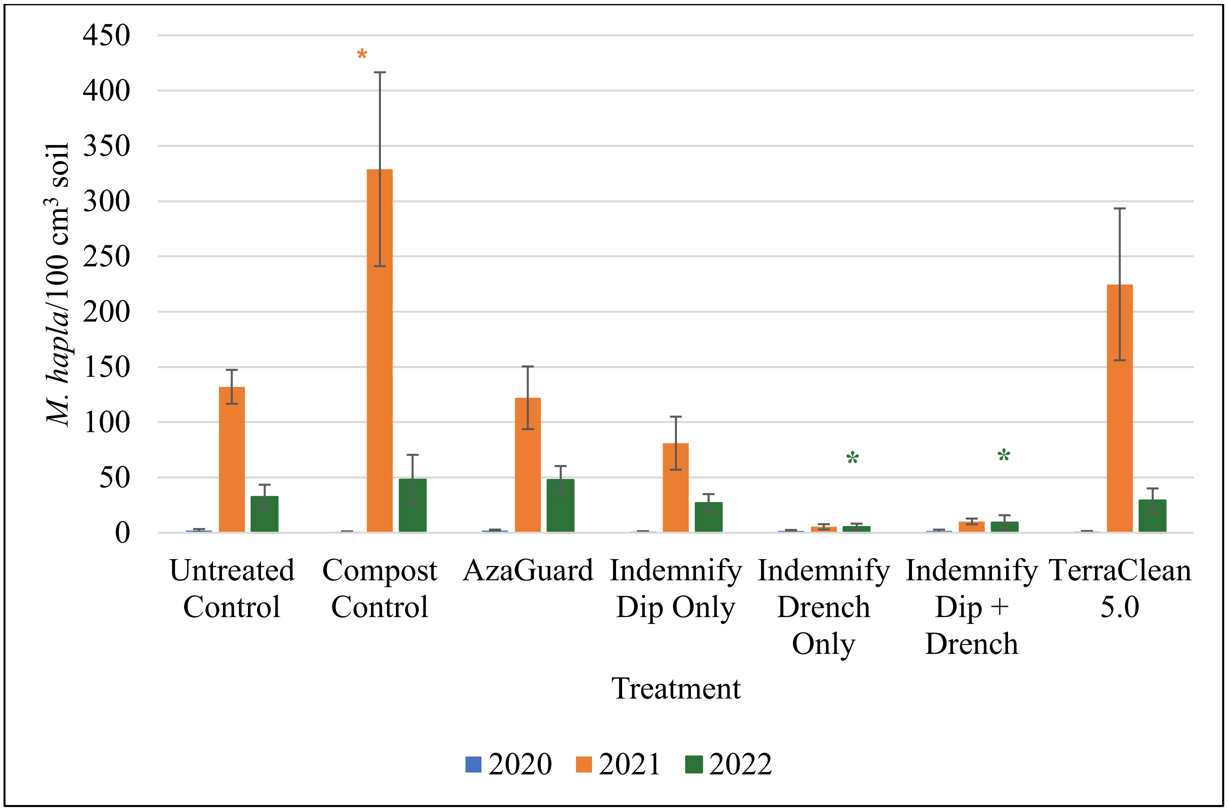
Table 3.4. Final mean *Hemerocallis* spp. measurements (N=5) of fresh shoot and root weights (g), crown width (cm), number of eyes/plant, and yield (G1s) at the end of the three-year dip field trial in October 2021. Means followed by the same letter within a column are not significantly different according to Tukey's honestly significant difference test ($P \leq 0.05$).

Treatments	Shoot Weight (g)	Root Weight (g)	No. of Eyes/ Plant	Crown Width (cm)	Yield (G1s)
Control	165.07 a	1302.87 a	11.80 a	15.93 a	1.87 a
Dip + AzaGuard	169.27 a	1397.87 a	13.27 a	16.13 a	2.40 a
Dip + 101 Starter Compost	197.33 ab	1644.67 a	14.13 a	16.00 a	2.60 a
Dip + Indemnify	198.93 ab	1456.53 a	12.00 a	15.40 a	2.80 a
Dip + TerraClean 5.0	220.40 b	1808.07 a	15.47 a	16.07 a	2.67 a
101 Starter Compost	195.20 ab	1742.60 a	12.60 a	18.80 a	1.80 a
***P*-values**	0.0284	0.117	0.680	0.670	0.380

3.3.2 High Carbon Compost Field Trial

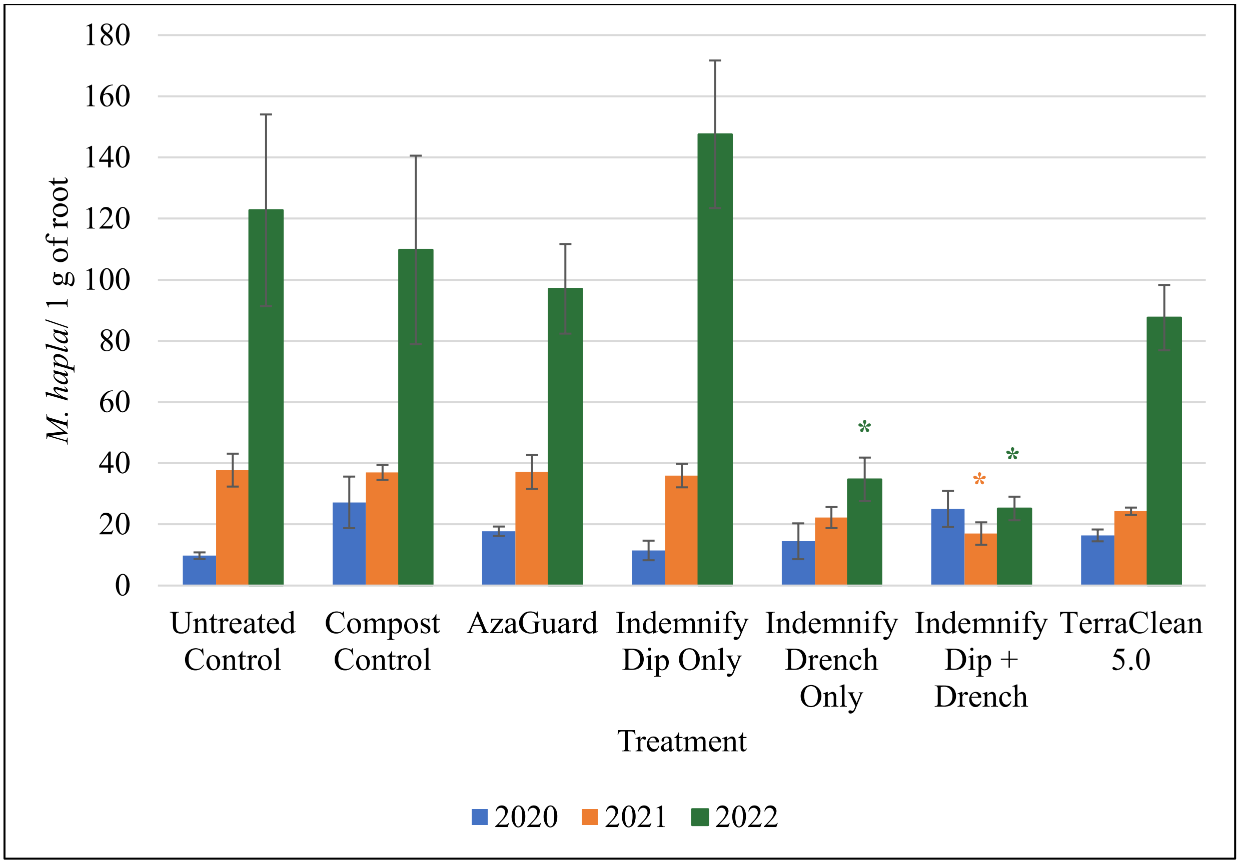
Throughout the duration of the compost field trial, the *M. hapla* population levels in the soil significantly differed all three years of the trial ($P \leq 0.05$) and when combined ($P < 0.001$). Overall, the Indemnify Drench Only treatment significantly had the lowest averaged population levels of 4.07 *M. hapla*/100 cm^3 soil (Figure 3.6), followed by the Indemnify Dip + Drench treatment with a population level of 7.00 *M. hapla*/100 cm^3 soil. The Compost Control had the highest population levels of 125.94 *M. hapla*/100 cm^3 soil. TerraClean 5.0 had the second highest population levels of 84.98 *M. hapla*/100 cm^3 soil, followed by AzaGuard with 57.26 *M. hapla*/100 cm^3 soil, and then the untreated control plots with an average population level of 55.44 *M. hapla*/100 cm^3 soil.

Figure 3.6. Mean ± SEM *Meloidogyne hapla*/100 cm^3 of soil for each treatment (N=5) in the compost field trial in 2020, 2021, and 2022. Asterisks indicate significant differences from the untreated control within the same year according to Tukey's HSD ($P \leq 0.05$).



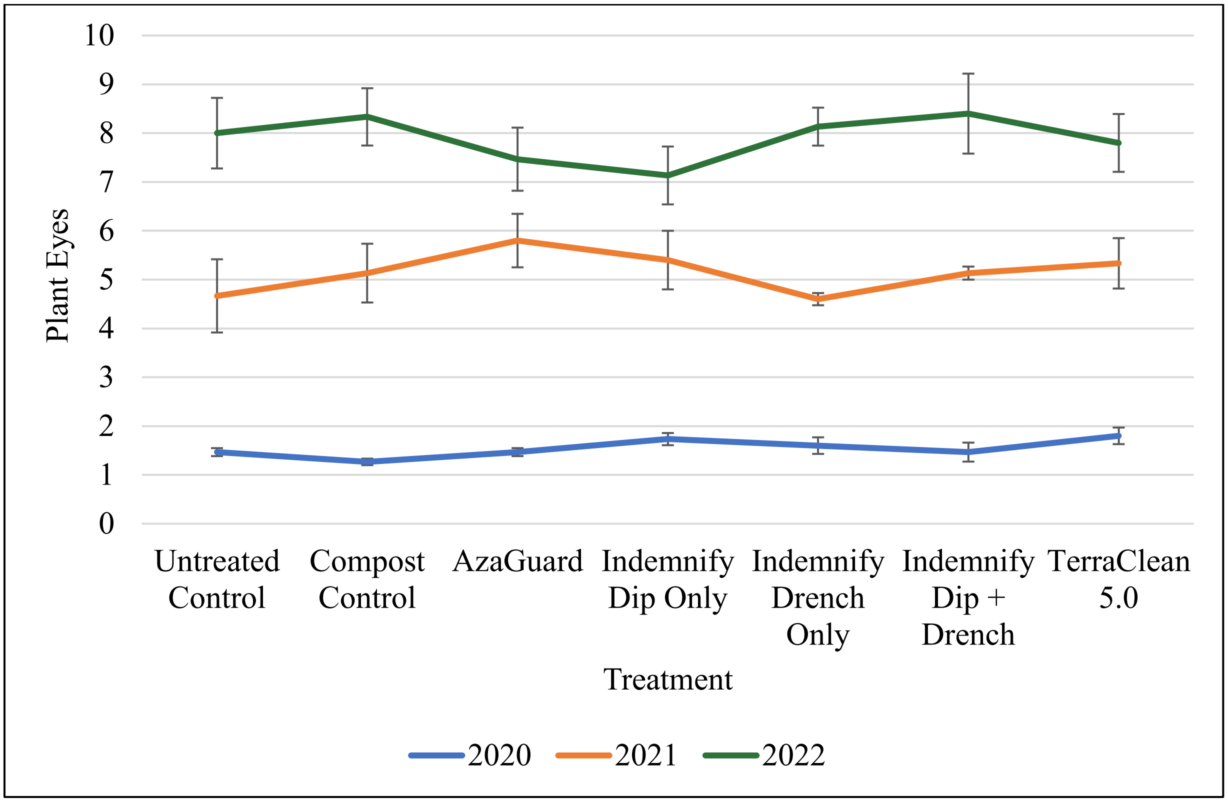
The population levels of *M. hapla* inside the roots (Figure 3.7) varied by treatment in 2021 ($P = 0.043$), in 2022 ($P < 0.001$), and over time ($P < 0.001$), but not in 2020 ($P = 0.125$). The root samples followed a similar trend to the soil population levels, with the Indemnify Dip + Drench and Indemnify Drench Only plants containing the lowest *M. hapla* population levels inside the roots of 22.44 *M. hapla*/1 g of root and 23.81 *M. hapla*/1 g of root, respectively. The Indemnify Dip Only treatment had the highest population level of 65.01 *M. hapla*/1 g of root, followed by the Compost Control and then the untreated control plants.

Figure 3.7. Mean ± SEM midseason *Meloidogyne hapla*/1 g of *Hemerocallis* spp. root for each treatment (N=5) in the compost field trial at midseason in July in 2020, 2021, and 2022. Asterisks indicate significant differences from the control (no compost) within the same year according to Tukey's HSD ($P \leq 0.05$).



Plant height measurements taken at midseason also significantly differed each year in the trial ($P \leq 0.05$) and when combined ($P \leq 0.001$) (data not shown). The Indemnify Drench Only plants significantly had the tallest plant heights of 38.33 cm, followed by the Indemnify Dip + Drench plants with an average height of 33.40 cm. The Indemnify Dip Only treatment had the smallest plant heights with an average height of 31.76 cm. The number of eyes/plant taken at the midseason sampling period significantly differed throughout the duration of the trial ($P < 0.001$) (Figure 3.8). The Indemnify Drench Only plants significantly had the most eyes/plant with an average of 5.00 eyes/plant, followed by TerraClean 5.0 with an average of 4.98 eyes/plant. The untreated control had the least amount of eyes/plant with an average of 4.71 eyes/plant.

Figure 3.8. Mean ± SEM midseason number of eyes/*Hemerocallis* spp. plant for each treatment (N=5) in the compost field trial at midseason in July in 2020, 2021, and 2022.



When the trial ended in October of 2022, three plants/plot were dug up to take final plant measurements on, such as shoot and root fresh weights (g), crown width (cm), final plant heights (cm), final number of eyes/plant, and yield (G1) (Table 3.5). The Indemnify Dip + Drench plants significantly had the largest shoot weights while the Indemnify Dip Only and the untreated control plants had the lowest. Though not significantly, the untreated control plants had the largest root system while the Indemnify Dip Only plants had the smallest. Looking at the crown width, the Indemnify Dip Only plants also had the smallest crown width, while the Indemnify Dip + Drench and Indemnify Drench Only plants numerically had the largest. The Indemnify Drench Only and Indemnify Dip + Drench plants significantly had the tallest plant heights compared to the rest of the treatments, with the Compost Control plants having the smallest plant heights. The Compost Control also had the least number of eyes/plant and the Indemnify Dip + Drench treatment had the highest number of eyes/plant, though not significantly. Lastly, yield also did not significantly differ among the treatments, but numerically, the Indemnify Dip + Drench plants had the highest yield while the untreated control plants had the lowest yield.

Table 3.5. Final mean *Hemerocallis* spp. measurements (N=5) of fresh shoot and root weights (g), crown width (cm), plant height (cm), number of eyes/plant, and yield (G1s) at the end of the three-year compost field trial in October 2022. Means followed by the same letter within a column are not significantly different according to Tukey's honestly significant difference test ($P \leq 0.05$).

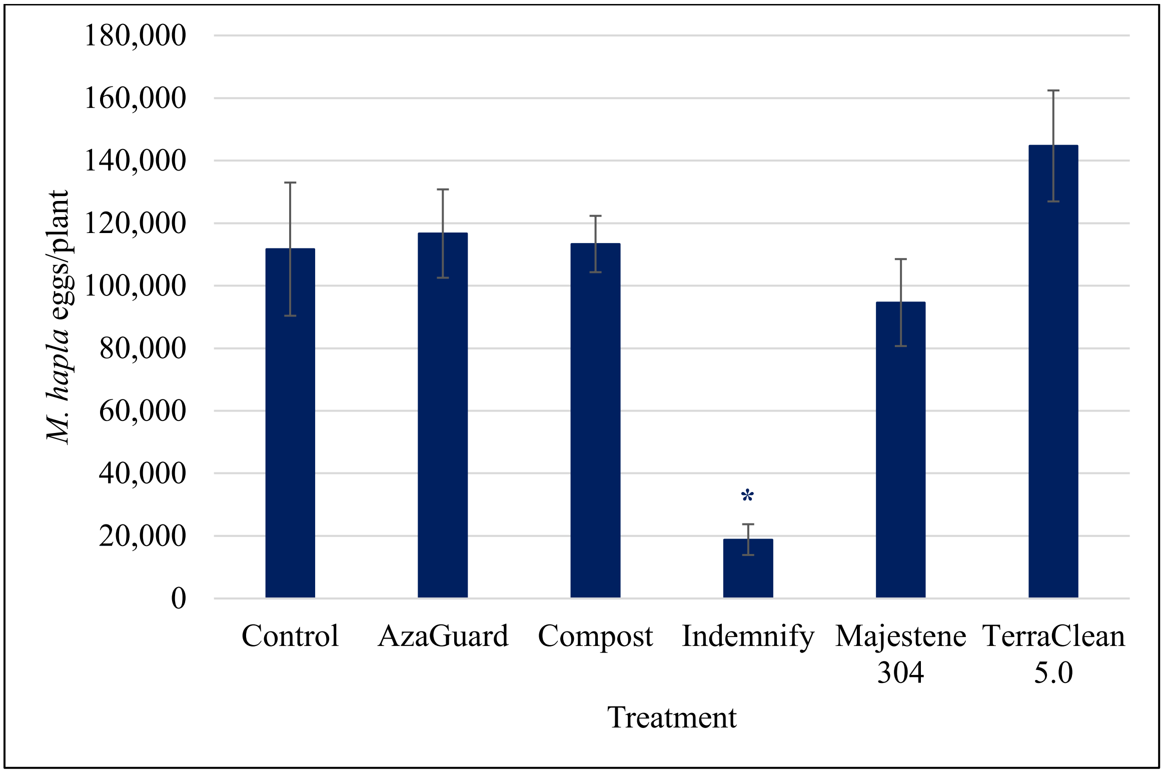
Treatment	Shoot Weight (g)	Root Weight (g)	Crown Width (cm)	Plant Height (cm)	No. of Eyes/ Plant	Yield (G1)
Untreated Control	124.60 a	1422.20 a	12.67 a	49.80 ab	9.00 a	5.47 a
Compost Control	141.60 ab	1289.20 a	12.60 a	45.00 a	8.07 a	6.20 a
AzaGuard	128.00 ab	1289.53 a	12.27 a	45.80 a	8.60 a	7.07 a
Indemnify Dip Only	123.13 a	1134.60 a	12.13 a	48.40 a	9.13 a	6.00 a
Indemnify Drench Only	204.93 ab	1286.27 a	14.20 a	54.67 b	8.80 a	7.87 a
Indemnify Dip + Drench	218.27 b	1194.33 a	15.20 a	54.27 b	10.40 a	8.60 a
TerraClean 5.0	163.40 ab	1166.93 a	12.93 a	48.67 a	8.80 a	6.67 a
***P*-values**	< 0.001	0.061	0.071	< 0.001	0.166	0.093

3.3.3 Alternative Methods Greenhouse Trial

In the greenhouse trial testing the top treatments from the three field trials, five treatments were applied to inoculated daylily plants in the spring of both years. After destructively harvesting the plants and extracting the *M. hapla* eggs from each individual root system (Figure 3.9), the Indemnify plants significantly had the lowest number of *M. hapla* eggs/plant with an average of 94,600 *M. hapla* eggs/plant ($P < 0.001$). TerraClean 5.0

significantly had the highest final population levels with 144,680 *M. hapla* eggs/plant. The gall ratings followed the trend of the *M. hapla* eggs/pot with Indemnify having the lowest gall rating of 0.58 galls/plant, but the Majestene 304 plants had the highest gall rating of 3.75 galls/plant followed by the control plants ($P < 0.001$).

Figure 3.9. Final mean ± SEM *Meloidogyne hapla* eggs/pot in the alternative methods greenhouse experiment. Values are the means of six replications. Asterisks indicate significant differences from the control within the same year according to Tukey's HSD ($P \leq 0.05$).



When the experiment was terminated in September 2022, differences were observed among the final plant measurements (Table 3.6). Majestene 304 significantly had the highest shoot weight of 164.67 g; the control plants had the lowest shoot weight of 77.00 g. Indemnify and AzaGuard significantly had the largest root weights with the control plants having the smallest root system. Numerically, the control plants also had the smallest crown width of 8.67 cm, with Majestene 304 having the largest crown width of 11.25 cm. The growth index did not significantly differ among treatments at the end of the trial; however, it did significantly differ over time ($P < 0.001$), with Indemnify having the largest growth index. Similarly, the final number of eyes/plant did not significantly differ at the termination of this experiment, but it did significantly differ over time ($P < 0.001$), with Indemnify and Majestene 304 plants having the most eyes/plant.

Table 3.6. Final mean *Hemerocallis* spp. measurements (N=6) of fresh shoot and root weights (g), crown width (cm), growth index (cm), and the number of eyes/plant at the end of the alternative methods greenhouse trial. Means followed by the same letter within a column are not significantly different according to Tukey's honestly significant difference test ($P \leq 0.05$).

Treatment	Shoot Weight (g)	Root Weight (g)	Crown Width (cm)	Growth Index (cm)[1]	No. of Eyes/ Plant
Control	77.00 a	587.83 a	8.67 a	70.08 a	6.17 a
AzaGuard	87.67 a	859.50 b	11.17 a	69.33 a	6.83a
Compost	84.17 a	710.17 ab	10.92 a	67.96 a	7.00 a
Indemnify	107.17 a	1010.33 b	9.92 a	71.29 a	7.33 a
Majestene 304	164.67 b	799.67 ab	11.25 a	78.58 a	7.33 a
TerraClean 5.0	93.33 a	810.67 ab	9.92a	67.63 a	7.00 a
***P*-values**	< 0.001	< 0.001	0.0718	0.783	0.534

[1]Growth Index (GI) values calculated as GI = (height + ((diameter 1 + diameter 2) / 2)) / 2.

3.3.4 *Paratylenchus* spp. Greenhouse Trial

In both years of the greenhouse experiment evaluating the host status of *Paratylenchus* spp. on *Hemerocallis* spp. plants and its impact on their growth, the final *Paratylenchus* spp. population levels/pot varied significantly ($P < 0.001$) with the inoculated daylily plants having the highest recovery of pin nematodes in both trials (Table 3.7). The corn plants, a known host, had the second highest recovery, and the non-inoculated daylilies and plain soil pots had none for both trials. Even though pin nematodes were recovered from both the corn and daylily pots, both plants had RF values close to 0 (Table 3.7), indicating they are not a host to *Paratylenchus* spp.

Table 3.7. Final mean *Paratylenchus* spp. population levels/pot and reproduction factor (RF) values of the daylily, corn, and the plain soil pots to determine the host status of *Paratylenchus* spp. on *Hemerocallis* spp. in two greenhouse trials. Values are the means of five replications. Means followed by the same letter within a column are not significantly different according to Tukey's honestly significant difference test ($P \leq 0.05$).

	Trial 1		Trial 2	
Inoculation Rate	**Final *Paratylenchus* spp./pot**	**RF[1]**	**Final *Paratylenchus* spp./pot**	**RF**
Daylilies Inoculated	147.63 b	0.016 b	102.21 b	0.011 b
Daylilies Non-Inoculated	0 a	0 a	0 a	0 a
Corn	56.78 b	0.006 ab	34.07 b	0.004 ab
Plain Soil	0 a	0 a	0 a	0 a
***P*-values**	< 0.001	< 0.001	< 0.001	< 0.001

[1]RF (Reproduction Factor) values calculated as final nematode population density/initial nematode population density.

When the trials were terminated after five months, final plant measurements were taken on the two daylily plant treatments. For the majority of the measurements, there were no differences observed between the inoculated and non-inoculated daylily plants (Table 3.8). However, in Trial 1, the growth index and crown width measurements were significantly different, with the non-inoculated daylily plants having a larger crown width and growth index. In Trial 2, there were significant differences observed in just the final number of eyes/plant, but the inoculated daylily plants had a higher amount compared to the non-inoculated daylily plants. Since there were no flower buds on any plant at the end of experiment for both Trial 1 and Trial 2, the number of flower buds were excluded from the analysis and data tables.

Table 3.8. Harvest fresh shoot and root weights (g), and final mean plant measurements of the *Hemerocallis* spp. plants to determine the host status of *Paratylenchus* spp. on daylily in two greenhouse trials. Values are the means of five replicates. Means followed by the same letter within a column are not significantly different according to Tukey's honestly significant difference test ($P \leq 0.05$).

	Trial 1						Trial 2					
Treatment	**Shoot Weight (g)**	**Root Weight (g)**	**Growth Index[1]**	**Crown Width (cm)**	**No. of Scapes/ Plant**	**No. of Eyes/ Plant**	**Shoot Weight (g)**	**Root Weight (g)**	**Growth Index**	**Crown Width (cm)**	**No. of Scapes/ Plant**	**No. of Eyes/ Plant**
Daylilies Inoculated	13.00 a	88.80 a	27.95 a	6.50 a	1.00 a	5.80 a	15.80 a	135.80 a	29.80 a	8.60 a	0.40 a	6.20 b
Daylilies Non-Inoculated	14.80 a	117.60 a	37.70 b	9.20 b	0.80 a	5.60 a	15.20 a	127.20 a	30.60 a	7.90 a	0.60 a	3.80 a
***P*-values**	0.478	0.061	0.035	0.012	0.694	0.992	0.762	0.602	0.663	0.559	0.681	0.034

[1]Growth Index (GI) values calculated as GI = (height + ((diameter 1 + diameter 2) / 2)) / 2.

3.4 DISCUSSION

With limited management strategies to combat plant-parasitic nematodes, alternative and more sustainable management strategies that can be applied throughout the ornamental plant production cycle are needed. The research trials in this dissertation chapter aimed to confirm new management systems to effectively control *M. hapla* in ornamental plant production fields and determine the impact and host status of *Paratylenchus* spp. on daylily plants. This was achieved through the conduction of two field trials at a commercial nursery in Zeeland, MI, and two greenhouse trials.

In the Dip Field Trial, the control plots had the lowest *M. hapla* population levels of both the soil and roots, but those plants also had the least number of eyes/plant and the lowest yield, followed by the 101 Starter Compost Only treatment. The Dip + Indemnify treatment had the highest population level of *M. hapla* inside the roots and the second highest soil population level; however, that treatment also resulted in plants with the tallest plant heights and the highest yield, indicating that Indemnify can significantly increase plant growth, regardless of nematode infection, to result in larger plants thereby leading to higher yields. With larger plants and therefore larger root systems, there are more places for nematodes to infect below ground resulting in higher *M. hapla* population levels. However, for this trial, it is important to keep in mind that a lot of the plant data was not significant, probably due to the fact that the plots were very small and on a slope, allowing treatments to possibly go beyond their plots and therefore not be as effective in their respective plots.

The results of the Compost Field Trial also had the Indemnify Dip Only plots resulting in significantly the highest *M. hapla* population levels inside the roots. The Indemnify Drench Only treatments and the Indemnify Dip + Drench treatment plants had both the lowest soil and root *M. hapla* population levels, indicating the importance of applying treatments every year of the daylily production cycle. The Indemnify Dip Only plants had no further applications of Indemnify beyond the one application in the first year of the trial, but the other two Indemnify treatments had it applied as a soil drench every spring. The benefit of applying treatments every year is also shown by the effect it had on plant growth. The Indemnify Drench Only plants significantly had the tallest plant heights and the most eyes/plant. Similarly, the Indemnify Dip +

Drench plants had the second tallest plant heights, the largest shoot weights, the largest crown width, and the highest yield, while the Indemnify Dip Only plants had the smallest midseason plant heights, the smallest shoot weights, the smallest root weights, and the smallest crown width. This impact on plant health is most likely directly related to *M. hapla* population levels; the Indemnify Drench Only and Indemnify Dip + Drench plots had the least amount of *M. hapla*/plot, while the Indemnify Dip Only plots had the most. This shows a clear impact on plant growth from *M. hapla* feeding. Howland et al. (2022) also found that the Indemnify soil drench and root dip applications were not similar in their control of *M. hapla*, with the Indemnify root dip plants having higher *M. hapla* population levels, indicating that applying Indemnify as a soil drench every year is crucial to more effective control of *M. hapla*.

These results also indicate that managing ornamental plants throughout the production cycle is essential to keeping *M. hapla* populations levels low enough for sufficient plant growth and is reinforced by the untreated control plant measurements. In the Compost Field Trial, the control plants had the lowest yield and shoot weights, and the third highest *M. hapla* population levels. Similarly, the Compost Control plants had the smallest plant heights and the least number of eyes/plant, which could be a result of those plots having the highest *M. hapla* population levels. Additionally, these results plus the results of the Dip Field Trial suggest that using only composts to try to manage nematode population levels in ornamental plant fields is not sufficient since those plots had some of the highest *M. hapla* population levels. This is further supported by the results of the Howland et al. (2022) first field trial where the composts did not effectively control *M. hapla* compared to the fumigated plots.

Looking at the other two treatments in both field trials, TerraClean 5.0 and AzaGuard, in the Dip Field Trial, the Dip + AzaGuard plots and the Dip + TerraClean 5.0 plots had the second and third lowest *M. hapla* population levels, respectively. The Dip + TerraClean 5.0 plants had the highest number of eyes/plant, the largest shoot and root weights, and the second highest yield by 42% compared to the control plants. In the Compost Field Trial, when the effects of these treatments were additionally tested with a compost, the Compost + Indemnify Dip + TerraClean 5.0 and Compost + Indemnify Dip + AzaGuard had the fourth and third highest *M. hapla* population levels, with a median number of eyes/plant and yield. Howland et al. (2022) found TerraClean 5.0, by itself, to be the most effective treatment in *M. hapla* management; that result in comparison to the higher population levels found in these two trials suggest that these two chemicals may be more effective by themselves than applying them in combination with other treatments such as an Indemnify dip.

When looking at the results of the third trial testing these treatments in a greenhouse setting, again, the Indemnify plants significantly had the lowest number of *M. hapla* eggs/plant by 83% compared to the control pots and the lowest gall rating, even though these plants had the largest root system. The Indemnify plants had an 80% reduction in galls on the root system compared to the control plant roots. These clear results support the results of the three field trials showing that Indemnify provides extremely effective management of *M. hapla* while reducing the number of galled roots and enhancing plant growth.

Surprisingly, the TerraClean 5.0 pots had the highest *M. hapla* population levels, perhaps because this chemical is for field-use only and therefore not as effective in a small greenhouse pot with daily watering. However, TerraClean 5.0 still resulted in a decrease in galled roots by 15% compared to the control plants. The Majestene 304 pots had the second lowest *M. hapla* population level, which supports the results of the first field trial (Howland et al. 2022), where Majestene 304 provided the second-best control of *M. hapla* in ornamental plant fields, but it did not result in a reduction of galled roots. In this greenhouse trial, the Majestene 304 and the Indemnify plants also had the largest plants with the highest growth indexes and number of eyes/plant over time and at the end of the experiment. The Majestene 304 plants also had the highest shoot weights and largest crown width. The AzaGuard pots had the second largest *M. hapla* population levels at the termination of this experiment and the second lowest yield; this result plus the results of the field trials suggests that AzaGuard may not be an effective management solution for *M. hapla* in ornamental plant fields.

Even though *Paratylenchus* spp. is found frequently in ornamental plant fields in Michigan, these nematodes are not included in management decisions, probably since the host status and potential impact these nematodes can have on ornamental plants is unknown. To date, no research has investigated the actual impact of this nematode on *Hemerocallis* spp. production, making these trials very important to the ornamental plant industry in Michigan. Fortunately, the results of this greenhouse trial show that *Hemerocallis* spp. is a very poor host, borderline non-host, to *Paratylenchus* spp. since the RF value and the final population levels were extremely low. Even though there were high population levels of *Paratylenchus* spp. found in the Dip Field Trial, the results of the pin greenhouse trial indicate that the daylily plants in the field may not be the host of this nematode. The weeds in the field may be the actual host to the *Paratylenchus* spp. present in these ornamental plant fields. However, since even the corn plant, a known host, had a low RF value, it suggests that the results may be inconclusive and further testing should be conducted. However, even if *Paratylenchus* spp. does feed on daylily plants, the greenhouse trial also suggests that they have a minimal to zero impact on daylily plants, since there was very little difference between the growth of the inoculated daylilies and non-inoculated daylilies.

In conclusion, these trials emphasize the importance of applying management strategies every year in the field production of ornamental plants, since *M. hapla* population levels increased every year of the production cycle with the third year having the highest population levels. Through the results of these studies, Indemnify as a soil drench by itself and in combination with it as a pre-plant dip was confirmed as the most effective management option to manage *M. hapla* population levels in ornamental plant fields. Majestene 304 and TerraClean 5.0 were also shown to be a promising treatment to control *M. hapla* in ornamental field production, while compost by itself is not an effective management solution for *M. hapla*. Lastly, the results of the greenhouse trial show that *Hemerocallis* spp. is not a host to *Paratylenchus* spp.; therefore, management decisions do not need to be focused on this nematode, but Indemnify with the 101 Starter Compost can provide effective control of *Paratylenchus* spp. in ornamental plant fields. Therefore, the results of these field and greenhouse trials provide effective new management systems to efficiently manage *M. hapla*, the number one pathogen in ornamental plant fields in northern North America, while increasing plant growth and yields to reduce the economic impact plant-parasitic nematodes can have in these high value fields.

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CHAPTER 4: DETERMINATION OF SOIL SUPPRESSION IN MONOCULTURE ORNAMENTAL PLANT FIELDS AGAINST THE NORTHERN ROOT-KNOT NEMATODE (*MELOIDOGYNE HAPLA*)

4.1 INTRODUCTION

The United States floriculture industry was valued at $6.43 billion in 2021 with Michigan being the third largest producer, producing 10% of all ornamental plants in the United States (USDA, 2022; USDA, 2021). The top categories within the floriculture industry are annual bedding/garden plants, potted flowering plants, foliage plants, herbaceous perennial plants, propagative material, and then cut flowers and cultivated greens. Michigan is the number one producer of annual bedding/garden plants with an economic value of $247.7 million dollars and is the second largest producer of herbaceous perennial plants with an economic value of $79 million dollars in 2020 (USDA, 2021).

In Michigan, several important herbaceous perennial plants, such as hosta, daylily, hibiscus, and astilbe, are grown in the field for two to three years before they are harvested and sold as premium plants. One of the main pathogens affecting field-grown ornamental plants in Michigan is the northern root-knot nematode, *Meloidogyne hapla* (Howland et al., 2022; Lindberg et al., 2018; LaMondia, 1996). *Meloidogyne* spp. are sedentary endoparasites, spending the majority of their lifecycle inside host plant roots. Due to their feeding, plants have reduced root systems, stunted roots and foliage, chlorosis, reduced winter hardiness, dieback in perennials, and they can predispose the plants to secondary infections by soil-borne pathogens and mites (Phani et al., 2021; Handoo, 1998; Inserra et al., 1998; Anwar and Van Gundy, 1989). High populations of *Meloidogyne* spp. can result in severe root decay and even plant death (Inserra et al., 1995). *Meloidogyne hapla* causes over 20% yield loss, prevents the sale of infected, symptomatic plants, and can quarantine entire fields since there is a zero-tolerance policy for *Meloidogyne* spp. (Howland et al., 2022; Lindberg et al., 2018). All of these can cause significant economic loss to the ornamental plant industry.

In the ornamental plant industry, there are two main traditional methods for controlling *M. hapla* population levels in the field: chemical fumigation and hot water dips. Both management methods focus solely on managing nematodes in the field at the beginning of the ornamental plant production cycle and not for the two- to three-year duration. Therefore, these main management strategies of *M. hapla* are not always successful (LaMondia, 1996). Additionally, fumigation is very expensive, toxic, and environmentally unsound. Reduction of pesticide usage and its impacts on the environment has become a priority in agricultural research (Van der Putten et al., 2006; Kerry, 1990). Therefore, determining new and sustainable management methods is crucial.

Biological control has shown to be an effective, alternative management method to harsh chemical nematicides and can be more stable with longer-lasting control (Agbenin, 2011). Biological control is where the ecosystem can be manipulated by using living organisms to suppress pest populations. There are hundreds of organisms shown to be antagonistic to plant-parasitic nematodes, such as fungi, bacteria, predatory free-living nematodes, mites, collembola, flatworms, and protozoa (Ferris and Jaffee, 2022; Agbenin, 2011; Kerry, 1990). For nematophagous fungi, there are several different ways that they can control nematodes, such as nematode-trapping fungi, endoparasitic fungi, toxin-producing fungi, and fungal parasites of eggs. Bacterial antagonists control nematodes through parasitism via adhesive spores, competition, and antibiosis by producing nematicidal compounds (AbdelRazek and Yaseen, 2020; Abd-Elgawad and Kabeil, 2012). The most abundant group of nematode parasites are fungi (Stirling, 1991), but the most researched microbial antagonist to plant-parasitic nematodes is the bacterium *Pasteuria penetrans* (Elhady et al., 2017).

The buildup of natural, antagonistic microbe communities in soil is common in long-term monoculture fields. Extensive monocultures may encourage beneficial and antagonistic microorganism populations to increase and become specific to the plant pathogens present in the soil. Therefore, specific suppression can be achieved during successive monoculture in fields over several years in response to the plant pathogens present (Silva et al., 2018; Raaijmakers and Mazzola, 2016; Berendsen et al., 2012). This is especially true for plant-parasitic nematodes, where fungal parasitism has been shown to be higher in monoculture fields as opposed to annual crop rotation fields (Chen, 2007).

For *Meloidogyne* spp., egg parasites are the most effective mechanism since nematode eggs are considered to be the most vulnerable state in the *Meloidogyne* spp. lifecycle (Viaene and Abawi, 1998). Numerous studies have investigated natural soil suppression against plant-parasitic nematodes in a wide range of agricultural crops, such as soybeans (Chen and Chen, 2002), sugarbeet (Westphal and Becker, 2001), vegetables (Verdejo-Lucas et al., 2002), and fruit trees (Stirling et al., 1979). However, to date, no studies have investigated nematode soil suppression occurring in ornamental plant fields, yet natural nematode suppression in monoculture ornamental plant production fields may be occurring.

While trying to find suitable locations for the field trials described in this dissertation at a commercial nursery in Zeeland, MI, surveys were conducted to determine *M. hapla* population levels. The surveys revealed that *M. hapla* population levels were unexpectedly low in the majority of the ornamental plant fields. This result led to the speculation that natural *M. hapla* suppression was occurring in these long-term, monoculture ornamental plant fields. Therefore, the aim of this study was to determine if real, natural suppression of *M. hapla* was the result of lower-than expected *M. hapla* population levels, and if so, its impact on *M. hapla* in conjunction with known effective nematicides.

4.2 METHODOLOGIES

To determine if suppressive soils were occurring in these long-term, monoculture ornamental plant fields and their effect on *M. hapla*, a greenhouse trial was established at the Michigan State University's Plant Greenhouses, East Lansing, MI. The trial was conducted from December 2021 until November 2022 using ornamental field soil collected pre- and post-fumigation with Telone II (1,3-dichloropropene; Corteva, Wilmington, DE). Monoculture ornamental plant fields were scouted to find plant-parasitic nematode-free soil, and soil was collected using a shovel in 5 gal buckets from the field on October 5, 2021. The field was fumigated with Telone II one day after the initial soil collection, and two weeks after that on October 20, 2021, more soil was collected in 5 gal buckets. The soil was kept at 10°C until use in the greenhouse trial. Soil was taken pre- and post-fumigation to determine if there were any actual antagonistic microorganisms in the soil that would no longer be present in the fumigated soil. Other studies have reported less suppression against plant-parasitic nematodes after fumigation (Kerry et al., 1980).

Nursery-grade bare-rooted *Hemerocallis* spp. cv. 'Going Bananas' plants (Walters Gardens, Zeeland, MI) were planted directly into either fumigated or non-fumigated soil in 3.7 L pots in the greenhouse. After two weeks, each pot was inoculated with 9,000 *M. hapla* eggs. The *M. hapla* inoculum was originally collected from a daylily field in Zeeland, MI, and reared on tomato (*Lycopersicon esculentum* Mill. cv. 'Rutgers;' Burpee Seeds, Warminster, PA). Inoculum was obtained by destructively harvesting six-month-old tomato plants and collecting eggs from the plant roots according to standard practices using a 10% NaOCl solution (Byrd et al., 1972; Hussey and Barker, 1973; Jenkins, 1964). Plants were inoculated by aliquoting the inoculum into four, 5-cm deep holes in the soil around the base of the plant and covered up with clean sand.

Two weeks after inoculation, the respective treatments were applied (Table 4.1). Treatments were applied according to label rate. Treatments chosen were based on the results of three field trials conducted at a commercial nursery in Zeeland, MI; the top two treatments were chosen, along with the high-carbon compost applied to all ornamental plant fields at that nursery and the fungicide Bravo Weather Stik. The fungicide was chosen since it targets and controls fungi in ornamental plant fields. All products were applied to the soil, even the fungicide, to determine their effect on both *M. hapla* and soil microorganisms.

Table 4.1. Characteristics and application rates of the treatments applied in the soil suppression *Hemerocallis* spp. greenhouse trial.

Treatments	**Manufacturer**	**Active Ingredient**	**Rate**
Indemnify	Bayer Environmental Science	Fluopyram	0.0042 ml/pot
Prescription Blend Compost	Morgan Composting Inc.	Composted dairy cow manure with wood ash	25.79 g/pot
Majestene 304	Marrone Bio Innovations	*Chromobacterium subtsugae*	18.14 g/pot
Bravo Weather Stik fungicide	ADAMA	Chlorothalonil	6.43 ml/pot
Untreated Control	--	Untreated Control	--

The experiment composed of eight replicates of each treatment times the two soil types for a total of 80 pots. Plants were arranged in a randomized block design in the greenhouse. The plants were kept at a 16h:8h light:dark photoperiod at 26°C and fertilized biweekly (15 ml/7.6 L, Peters' Professional 20-10-20 N-P-K, ICL Specialty Fertilizers, Dublin, OH). Plant measurements were taken biweekly to determine the effect of the treatments and *M. hapla* infection on daylily plant growth. Plant measurements recorded were plant height (cm), number of eyes, number of flower buds, and number of scapes. We additionally measured width diameter (cm) N-S and E-W to generate a growth index (Krug et al., 2010).

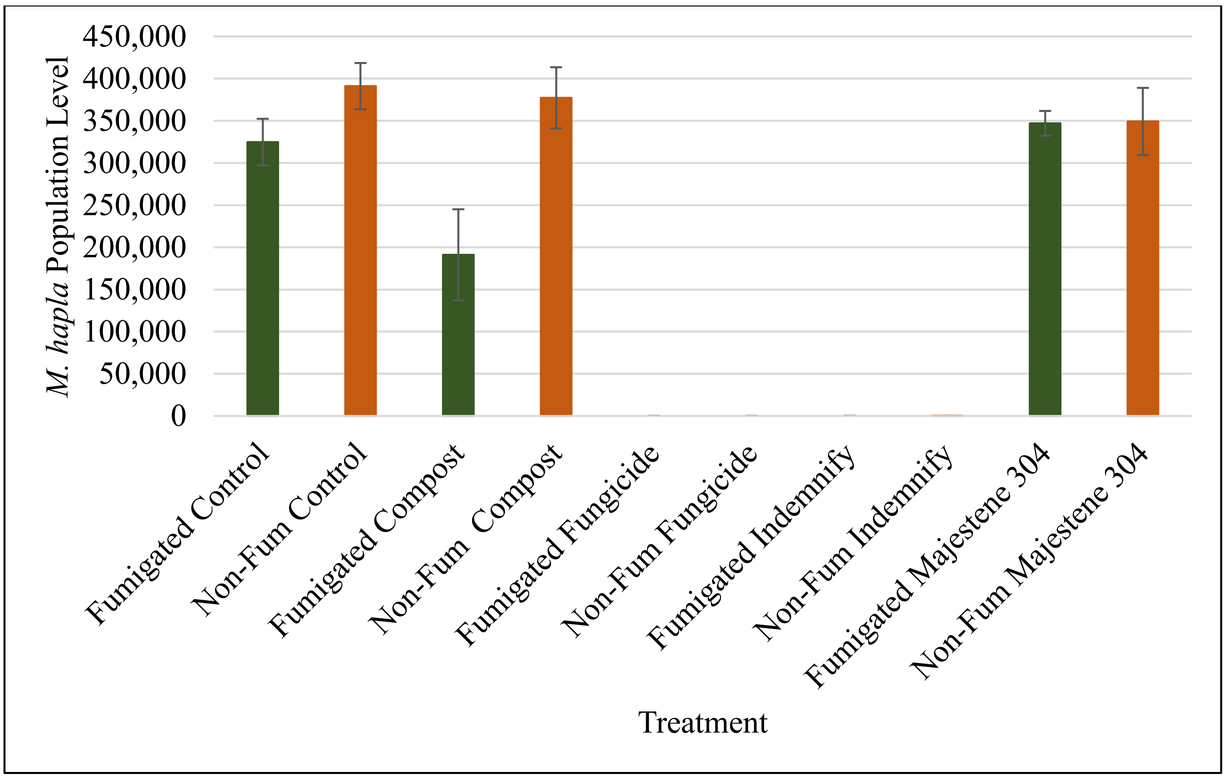
At the end of the experiment, final plant parameter measurements were taken (final plant height and diameter measurements, number of eyes, number of flower buds, and number of scapes), and then each individual plant was destructively harvested to obtain shoot and root fresh weights (g). A 250 cm^3 soil sample was collected from each respective pot to determine beneficial nematode population levels and a *M. hapla* gall rating was conducted on a numeric scale of 0 to 5 where 0 = 0 galls, 1 = 1 to 2 galls, 2 = 3 to 10 galls, 3 = 11 to 30 galls, 4 = 31 to 100 galls, and 5 = > 100 galls per root system (Taylor and Sasser, 1978). The entire root system was then placed in a Ziploc bag (SC Johnson, San Diego, CA) to determine final *M. hapla* population levels by extracting the *M. hapla* eggs/pot according to standard protocols (Byrd et al., 1972; Hussey and Barker, 1973; Jenkins, 1964). Briefly, roots were placed in a 500 ml covered container with a 10% NaClO solution and shaken for four minutes on a lab benchtop shaker (New Brunswick Scientific Co., Inc., Edison, NJ) to free *M. hapla* eggs from the gelatinous matrix they reside in. After the roots were shaken, the solution was poured over nested 250-µm and 25-µm sieves and rinsed for 30 seconds. The eggs retained on the 25-µm sieve were backwashed into a 15 ml polyethylene tube. *Meloidogyne hapla* eggs from each respective plant were then enumerated on an inverted microscope.

Data from the greenhouse trial was analyzed in R 4.0.3 (R Core Team, 2020). Data distribution was assessed before analysis and was log10(x + 1) transformed to meet normality assumptions, if needed. Analysis of variance (ANOVA) was used followed by means separation to determine if there was a significant difference among the treatments for *M. hapla* data, beneficial nematode data, and plant measurement data for fumigated soil, nonfumigated soils, and combined. Repeated measures analysis using linear regression was also conducted on the biweekly plant evaluations taken throughout the duration of the greenhouse trial, such as the growth index measurements, number of eyes, number of flower buds, and number of scapes. Tukey's honest significance difference test ($P \leq 0.05$) was used to determine differences among treatments in the 'emmeans' package in R (Lenth, 2019).

4.3 RESULTS

In the greenhouse trial evaluating the effect of potential biological controls on *M. hapla,* the population levels of *M. hapla* differed significantly across treatments ($P < 0.001$) in both fumigated and non-fumigated soils (Figure 4.1). The non-fumigated soil pots had on average 223,570 *M. hapla* eggs/pot while the fumigated soil pots had on average 172,655 *M. hapla* eggs/pot. The non-fumigated control had the overall highest *M. hapla* population level of 391,160 *M. hapla* eggs/pot. Analyzing the two soils separately, *M. hapla* population levels also differed ($P < 0.001$) within the fumigated soil and within the non-fumigated soil pots by treatment. Within the fumigated soil, the Majestene 304 treatment, followed by the control pots, had the highest population levels of *M. hapla*; the Bravo fungicide treatment, followed closely by the Indemnify treatments, had the lowest population levels. Within the non-fumigated soil, the control pots had the highest *M. hapla* population level, and similar to the fumigated soil pots, the Bravo fungicide, followed closely by the Indemnify treatments, had the lowest population levels of 40 *M. hapla* eggs/pot and 50 *M. hapla* eggs/pot, respectively. Similarly, *M. hapla* galls significantly differed when the two soils were analyzed together and separately ($P \leq 0.001$) and followed the same trends as the *M. hapla* population levels.

Figure 4.1. Final *Meloidogyne hapla* population levels ± SEM by treatment in fumigated and non-fumigated (non-fum) soils.



Looking at the population levels of the nematodes recovered from the soil from each pot, the nematodes differed by treatment ($P < 0.001$; Table 4.2). The non-fumigated soil had the highest recovery of beneficial nematodes (bacterivores, fungivores, and predators), with bacterivores being the predominant group. The non-fumigated control had the highest mean level of 984 bacterivores/pot, while the non-fumigated Indemnify, fumigated Bravo fungicide, and fumigated Indemnify had the lowest with an average of 6 bacterivores/pot. The non-fumigated compost followed by the non-fumigated Majestene 304 had the highest levels of fungivores/pot. There were no predators found in any of the pots in either soil type. Infective stage *M. hapla* J2 were also found in the soil and followed the trend of the *M. hapla* eggs/pot.

Table 4.2. The mean final population levels of *Meloidogyne hapla* J2s, bacterivores, fungivores, and predators found in the soil for each treatment in the soil suppression *Hemerocallis* spp. greenhouse trial. Means followed by the same letter within a column are not significantly different according to Tukey's honestly significant difference test ($P \leq 0.05$).

Treatment	*M. hapla* J2	Bacterivores	Fungivores	Predators
Fumigated Control	216 d	504 f	12 c	0 a
Fumigated Compost	24 b	324 d	0 a	0 a
Fumigated Bravo Fungicide	0 a	6 a	0 a	0 a
Fumigated Indemnify	0 a	6 a	0 a	0 a
Fumigated Majestene 304	48 c	240 c	12 c	0 a
Non-fumigated Control	264 e	984 g	12 c	0 a
Non-fumigated Compost	336 f	396 e	60 e	0 a
Non-fumigated Bravo Fungicide	0 a	66 b	6 b	0 a
Non-fumigated Indemnify	0 a	6 a	0 a	0 a
Non-fumigated Majestene 304	48 c	396 e	36 d	0 a
***P*-values**	< 0.001	< 0.001	< 0.001	0.461

Across the whole trial, trends were similar in both fumigated and non-fumigated pots with Bravo fungicide plants resulting in the lowest plant growth index ($P < 0.001$; Table 4.3). The pots with the significantly highest growth index were the fumigated Majestene 304 plants with a growth index of 46.25 cm ($P < 0.001$). The fumigated pots showed initial higher plant growth compared to the non-fumigated pots and that trend continued throughout the trial with the fumigated pots having higher plant growth indexes than the non-fumigated pots. Plant height measurements also differed over time ($P < 0.001$), with the Bravo fungicide treatment having the smallest plant heights in both soils and the Indemnify pots having the tallest plants in both soil types (data not shown).

The number of scapes ($P < 0.001$) and flower buds ($P < 0.001$) also differed over time with the Indemnify treatment resulting in plants having the most in both soils; the Bravo fungicide treatments had the lowest (data not shown). Both shoot and root fresh weights (g) differed according to treatment ($P \leq 0.001$) with the non-fumigated pots having lower shoot and root weights compared to the fumigated pots (Table 4.3). Within each soil type, Majestene 304 had the highest shoot weight, and the non-fumigated Bravo fungicide had the lowest shoot weight. The fumigated compost had the overall largest root weight and again, the non-fumigated Bravo fungicide had the lowest. Lastly, crown width (cm) significantly differed ($P < 0.001$) with the fumigated pots having larger crown widths compared to the non-fumigated pots, with the non-fumigated control plants having the smallest crown widths of 8.38 cm and the fumigated Majestene 304 having the largest crown widths of 10.94 cm.

Table 4.3. Final mean *Hemerocallis* spp. measurements (N=8) of growth index (cm), fresh shoot and root weights (g), and crown width (cm) at the end of the soil suppression greenhouse trial. Means followed by the same letter within a column are not significantly different according to Tukey's honestly significant difference test ($P \leq 0.05$).

Treatment	Growth Index[1] (cm)	Shoot Weight (g)	Root Weight (g)	Crown Width (cm)
Fumigated Control	39.97 ab	43.63 a	948.63 cd	10.31 ab
Fumigated Compost	37.91 ab	35.50 a	984.00 d	10.13 ab
Fumigated Bravo Fungicide	30.88 a	37.38 a	541.63 ab	8.81 a
Fumigated Indemnify	38.50 ab	46.25 a	821.13 bcd	10.25 ab
Fumigated Majestene 304	46.25 b	85.75 b	590.13 ab	10.94 b
Non-fumigated Control	34.09 ab	35.63 a	564.88 ab	8.38 a
Non-fumigated Compost	38.41 ab	35.50 a	631.38 abc	8.69 a
Non-fumigated Bravo Fungicide	32.09 a	31.38 a	388.63 a	9.69 ab
Non-fumigated Indemnify	30.53 a	33.75 a	857.00 bcd	8.94 a
Non-fumigated Majestene 304	31.63 a	44.63 a	523.63 ab	10.13 ab
***P*-values**	< 0.001	< 0.001	< 0.001	0.0003

[1]Growth Index (GI) values calculated as GI = (height + ((diameter 1 + diameter 2) / 2)) / 2.

The number of eyes, which is indicative of yield, significantly differed over time ($P < 0.001$) with the Bravo fungicide plants having the overall lowest number of eyes in both fumigated and non-fumigated pots (Figures 4.2 and 4.3). The control plants had the second lowest number of eyes. The Majestene 304 pots had the highest number of eyes in both soils. At the termination of the experiment, yield significantly differed ($P < 0.001$) across the whole trial and within the two soil types. Following the plant growth parameter's trends, the fumigated soil plants had a higher yield than non-fumigated soil plants, but the non-fumigated Majestene 304 pots had the overall highest yield, followed by the Indemnify pots. Lastly, performing a simple comparative analysis between the two soil types, showed that the fumigated soil pots had a significantly higher growth index, shoot and root weights, and crown width compared to the non-fumigated soil ($P \leq 0.05$); the number of eyes was also higher in the fumigated pots, though not significantly ($P = 0.145$).

Figure 4.2. The mean number of *Hemerocallis* spp. eyes (yield) ± SEM in the fumigated soil pots throughout the duration of the soil suppression greenhouse experiment.

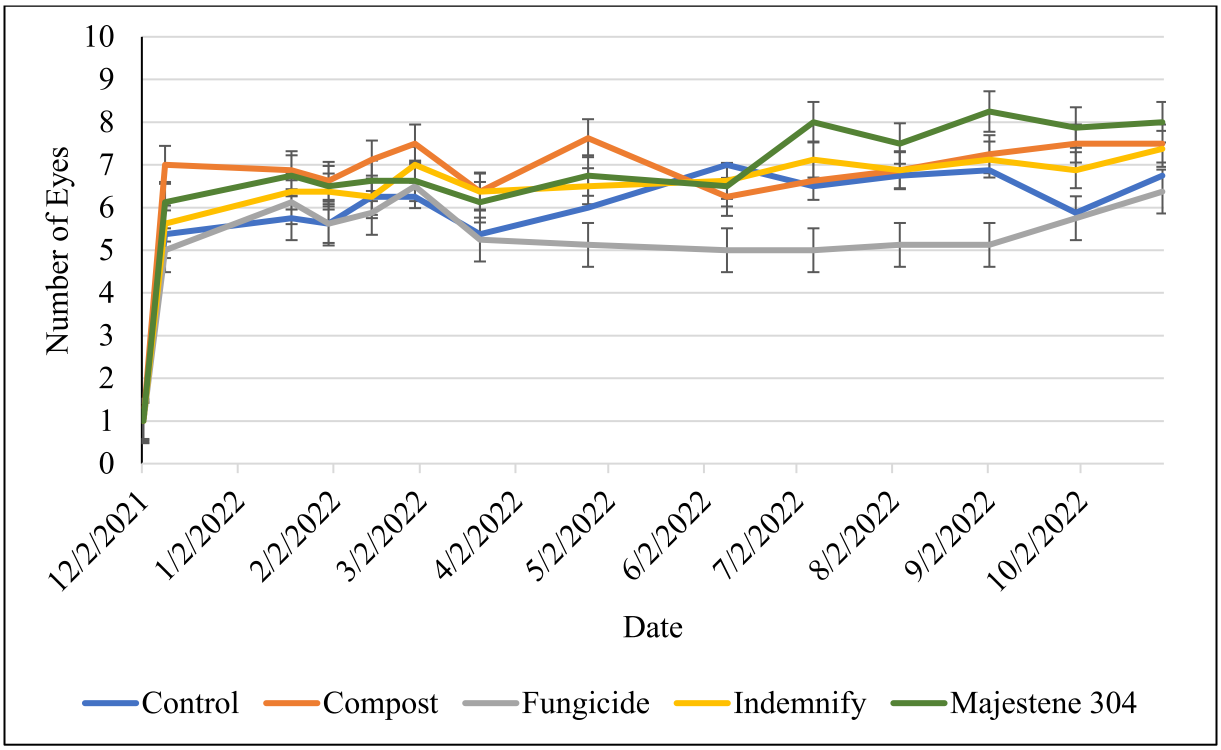
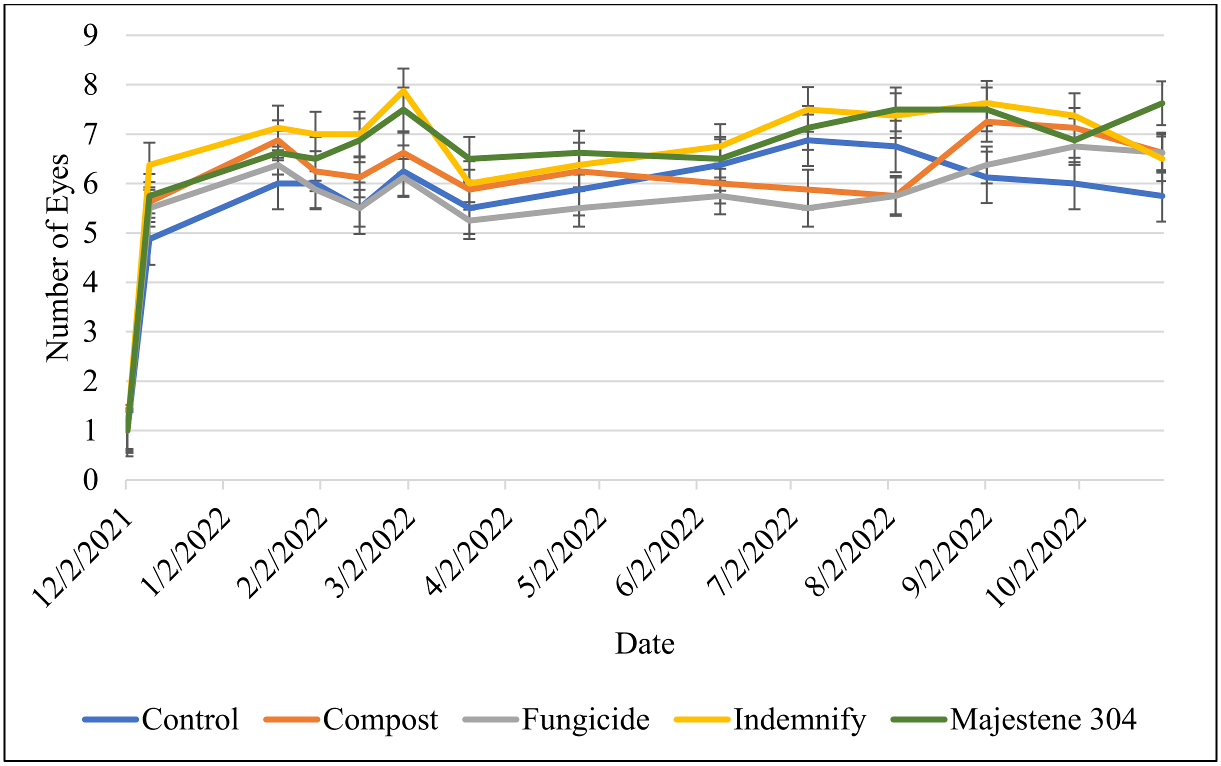


Figure 4.3. The mean number of *Hemerocallis* spp. eyes (yield) ± SEM in the non-fumigated soil pots throughout the duration of the soil suppression greenhouse experiment.



4.4 DISCUSSION

A greenhouse experiment was conducted to determine the effectiveness of nematicides, the effect of fumigation, and the impact of biological control on *M. hapla* in monoculture ornamental plant fields in Michigan. The pots containing the fumigated soil had overall lower *M. hapla* population levels and gall ratings compared to the non-fumigated pots, suggesting that it is unlikely that any suppression of *M. hapla* is occurring in this long-term ornamental plant field. Unfortunately, the results of this experiment contradict the findings from most studies where fumigated soils resulted in decreased soil suppression leading to higher plant-parasitic nematode population levels (Kerry et al., 1980). In a similar study, Adam et al. (2014) investigated three suppressive agricultural soils on *M. hapla* and found that *M. hapla* had significantly less galls, egg masses, and eggs in those soils when compared to sterilized soils, again contracting our results.

A possible explanation for this outcome is that these ornamental plant fields have a history of continual fumigation. In Michigan ornamental plant fields, fields are fumigated, regardless of pest pressure, every couple of years. As a result of this, it is possible that beneficial and nematode-antagonistic microorganisms have not had time to become reestablished in these fields. Therefore, even though these fields have a long-term ornamental plant production history, there is an inability to naturally suppress pathogens due to their frequent fumigation. Repeating this study in monoculture ornamental plant fields without a history of fumigation needs to be conducted to determine if true, natural suppression may be occurring in ornamental plant fields. Additionally, testing if adding composted manure or soil from another field can speed-up the reestablishment of beneficial microbes to aid in the natural suppression of pests could add great insight to plant-parasitic nematode management in these fields.

Examining the effect of treatments on *M. hapla*, Indemnify nematicide was found to be highly effective against *M. hapla*, which supports other studies that also found it to be effective against *Meloidogyne* spp. (Howland et al., 2022; Nnamdi et al., 2022; Dahlin et al., 2019; Ross, 2016). Majestene 304 had similar population levels to the control pots suggesting this bionematicide was not effective against *M. hapla* in this experiment, even though another ornamental plant study found Majestene 304 to be very effective (Howland et al., 2022). The differing environments (field verses greenhouse) and soil types used in Howland et al. (2022) and this experiment could help indicate why the results differed among the two experiments. Although Majestene 304 had the highest *M. hapla* population levels and the smallest root systems, it had the highest yield and crown widths. This further supports what Howland et al. (2022) found, which was that daylily seems to be tolerant to *M. hapla* infection.

The Bravo fungicide treatment resulted in plants with the lowest *M. hapla* population levels and some of the lowest beneficial nematode population levels. This is probably due to the fact that the fungicide was applied to the soil, and not to the foliage. Since it is a contact fungicide, it was applied to the soil to kill any soil-borne fungi to see if a fungus was the biological control source. Even though Bravo is a foliage fungicide meant for field use, it was chosen due to its frequent use in ornamental plant fields in Michigan and chlorothalonil, its active ingredient, is one of the most commonly used fungicides (Baćmaga et al., 2018). In this experiment, the fungicide also resulted in daylily plants with the smallest plant biomasses, growth indexes, and yields. Fungicides' negative impact on plant physiology is well documented (Baćmaga et al., 2018; Choate et al., 2013; Dias, 2012), causing plant stunting and phytotoxicity, which was observed in this trial. However, in ornamental plant fields, stunting as a result of fungicide application is negligible since the plants have a three-year production cycle.

The beneficial nematode population levels where higher in the non-fumigated soils compared to the fumigated soils, as expected. Since there were no predators found in any of the pots in either soil type, it suggests that predatory nematodes are not helping build antagonistic soils in ornamental plant fields. However, more fields should be screened for potential natural soil suppression. Only one field was studied in this trial, yet the field production acreage of ornamental plants is huge. An additional important next step would also be to conduct a metagenomic study of the soil microorganisms to determine what microbes exist in these monoculture ornamental plant fields. Isolating fungi or bacterial from *M. hapla* nematodes and from soil samples and conducting PCR of fungal ITS DNA or bacterial 16S rRNA to determine their species would determine if the fungi or bacteria found in these fields are known antagonistic organisms against *M. hapla*.

In conclusion, even though natural soil suppression in this ornamental plant field seems unlikely, natural *M. hapla* suppression may be occurring in long-term monoculture fields with no fumigation history. Additional trials investigating natural suppression against *M. hapla* in more than one field needs to be conducted. Further studies will provide great insight and can be used to develop effective management strategies for *M. hapla*. Lastly, this experiment showed that Indemnify was highly effective in managing *M. hapla* population levels giving ornamental plant producers a valuable management option to combat *M. hapla* in ornamental plant production fields and greenhouse plants.

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CHAPTER 5: CONCLUSION

The United States floriculture industry was valued at $6.43 billion in 2021 with the largest producers in the ornamental plant industry being Florida, California, Michigan, New Jersey, and Ohio (USDA, 2022). Michigan is the third largest producer, producing 10% of all ornamental plants in the United States (USDA, 2021). It is also the number one producer of annual bedding/garden plants with an economic value of $247.7 million dollars, and is the second largest producer of herbaceous perennial plants with an economic value of $79 million dollars in 2020 (USDA, 2021).

Within the herbaceous perennial plants, daylily (*Hemerocallis* spp.) production is a major component, with an economic value of $16.8 million in 2020 (USDA, 2021). Daylily is one of the most popular and important ornamental perennial plants in landscapes and gardens (Mosonyi et al., 2019; Gulia et al., 2009; Gatlin, 1999) and has a wide range of uses such as for food, medicine, beautification, and environmental conservation such as preventing soil erosion (Wali et al., 2022; Munson, 1989). Daylilies are widely cultivated, with approximately 20 species and over 98,000 registered cultivars in the United States (American Daylily Society, 2023; Gulia et al., 2009). Since these plants are grown in the field for several years, daylilies can be plagued by numerous pathogens making the production of clean plant material a challenge, especially due to plant-parasitic nematodes. In Michigan, plant-parasitic nematodes cause millions of dollars in economic loss each year in the state's $104.7 billion agriculture industry (Bird and Warner, 2018). In ornamental plant fields, the northern root-knot nematode, *Meloidogyne hapla*, is the most economically important perennial ornamental pathogen in the northern United States and Canada, causing over 20% yield loss in daylily production (Howland et al., 2022; Lindberg et al., 2018; LaMondia, 1996).

Meloidogyne spp. are the most economically important plant-parasitic nematodes due to their worldwide distribution and large host range of over 3,000 plant species (Abad et al., 2003). Root-knot nematodes are sedentary endoparasites remaining stationary inside the roots of a host plant with the plant growing around them to form galls. Due to the presence of these galls on ornamental plant roots, they prevent the sale of infected, symptomatic plants, and can quarantine entire fields since plant exports are inspected before field harvest and shipping. Despite this, nematodes can still be easily spread, especially through asymptotic plants. Even though *Meloidogyne* spp. are widespread, their ability to build up their populations so rapidly in the soil and our lack of complete management, make preventing their spread so important. Therefore, there is a zero-tolerance policy for *Meloidogyne* spp. on plant exports (Howland et al., 2022; Lindberg et al., 2018).

Another plant-parasitic nematode commonly found in Michigan ornamental plant fields are pin nematodes, *Paratylenchus* spp. (Howland et al., 2022). Unlike root-knot nematodes, pin nematodes are migratory ectoparasites that feed on the exterior surfaces of host plant roots. However, these nematodes are not included in management decisions, probably since the host status and potential impact these nematodes can have on ornamental plants is unknown. In fact, for both plant-parasitic nematodes, the damage potential and threshold levels are unknown, which can lead to poor management decisions.

Nematodes are extraordinarily difficult to manage and almost impossible to eradicate since they can remain in the soil for many years without a host. Current management strategies for *M. hapla* in ornamental field production are limited to two main options: hot water dips and preplant fumigation. Hot water dips can be effective; however, they can cause up to 50% mortality of the propagules and can drastically reduce vigor. Preplant soil fumigation is very effective in annual production systems, but in ornamental plant fields that are in production for several years, fumigation only controls nematodes in the first year and provides only 60-70% control of *M. hapla* in Michigan ornamental production fields. Neither of these management options control plant-parasitic nematodes throughout the whole daylily production cycle. This drastically emphasizes the need for better management options of plant-parasitic nematodes in ornamental production fields that control nematode populations throughout the entire growing season.

Therefore, the goal of this dissertation was to determine more effective management strategies for *M. hapla* in Michigan ornamental plant fields to prevent yield loss and exportation rejection, and reduce the economic burden of this pest. This goal was successfully accomplished through three objectives: 1) Determine alternative management strategies to control *M. hapla* in daylily production fields; 2) Evaluate the host status of *M. hapla* and *Paratylenchus* spp. on daylily production in the greenhouse; and 3) Determine the production impact and action thresholds of *M. hapla* and *Paratylenchus* spp. on daylily production in the greenhouse.

These objectives were accomplished through the conduction of three, three-year field trials at a commercial nursery in Zeeland, MI, and four greenhouse experiments at Michigan State University's Plant Greenhouses, East Lansing, MI. The field and greenhouse trials tested many different management options and combination of management options to find new management strategies to control *M. hapla* in ornamental plant fields and reduce the formation of galls on plant roots. Treatments included were Indemnify nematicide used both as a preplant dip and as a soil drench application, three compost manures, Advanced Ag bionematicide, AzaGuard bionematicide, Majestene 304 and 305 bionematicides, TerraClean 5.0 nematicide, combinations of Indemnify as a preplant dip with other treatments, and combinations of treatments with a high-carbon compost. Two other greenhouse trials tested the host status of *Hemerocallis* spp. to *M. hapla* and *Paratylenchus* spp., and the damage potential of both nematodes on daylily plants; the threshold of *M. hapla* was also determined.

The results of these multi-year studies demonstrate that there are more effective solutions for *M. hapla* management in ornamental plant production fields compared to the current management practices, such as fumigation. The results suggest three new management options for reducing *M. hapla* population levels in ornamental plant fields and reducing the presence of *M. hapla* galls on plant roots. Overall, Indemnify as a soil drench by itself, Majestene 304, and TerraClean 5.0 have been shown to provide the best *M. hapla* management in daylily fields when applied annually. While Indemnify as a drench plus preplant dip did also show promising *M. hapla* management, having a dip is one additional step, cost, and potential time constraint in a management plan. Indemnify as a soil drench by itself was shown to be more effective than combining it with the preplant dip. Indemnify as a soil drench decreased *M. hapla* population levels by an average of 39.5% compared to the control plots of no treatment, and decreased the population levels 7.5% compared to the fumigated treatment. Additionally, it reduced the number of galls by 80% compared to the control plants, which is considerable and can lead to less fields being quarantined and fewer shipment rejections, significantly increasing the profits of the ornamental plant industry. Majestene 304 and TerraClean 5.0 reduced *M. hapla* population levels by an average of 34.7% and 28.8%, respectively. TerraClean 5.0 also had a reduction in the number of galled roots by 15%; Majestene 304 did not have a decrease in galls on the plant roots compared to the control plant roots.

In addition to the Indemnify treatment providing the best *M. hapla* control, it was also shown to have a positive effect on plant growth, producing plants with some of the largest overall plant biomass, such as plant heights, shoot weights, crown widths, and, most importantly, yield. Plants where Indemnify was applied as a soil drench always had higher yields (on average 41.3% higher) compared to the control plants and higher yields (on average 40% higher) compared to Telone II fumigation. Most importantly, these alternative management strategies can not only provide better *M. hapla* control and boost plant growth due to less pest pressure, but they are much more cost effective compared to preplant fumigation, which is very expensive. These three options therefore provide the ornamental plant industry much more attractive, cost effective, and efficient management plans for *M. hapla*.

These experiments also show that, unlike some agricultural crops such as potatoes (Cole et al., 2020), compost by itself is not an effective management solution for nematodes in ornamental plant fields. While compost by itself can produce larger plants compared to the control plants, it did not always keep *M. hapla* population levels low, and therefore, it should be used in conjunction with other treatments. The results from these trials also show that applying treatments throughout the production cycle is crucial and provides significantly better *M. hapla* management compared to current practices. In all of these trials, both greenhouse and field, treatments were applied every year in the spring. Since *M. hapla* populations increased each year of the production cycle, yearly treatment applications are essential to help prevent this population build up. Most importantly, these trials show that there was no impact on plant growth, health, and yield from annual treatment applications. Therefore, annual treatment applications in the ornamental plant production cycle is a new management strategy that can effectively reduce *M. hapla* population levels in these high value fields while having no impact on plant performance.

The greenhouse trials to determine the host status of *M. hapla* and *Paratylenchus* spp. on *Hemerocallis* spp. indicate that daylily is an excellent host to *M. hapla*, with reproduction factor (RF) values ranging from 1.82 to 1365.66. The greenhouse trial testing the same *Hemerocallis* spp. cultivar on *Paratylenchus* spp., showed that these daylily plants are an extremely poor host to pin nematodes, with RF values ranging from 0.011 to 0.016. Additionally, the pin nematode greenhouse trial suggests that they have a minimal to zero impact on daylily plants, since there was very little differences in the growth between the inoculated daylilies and the non-inoculated daylilies. This is excellent news for the ornamental plant industry and indicates that even though *Paratylenchus* spp. can be frequently found in ornamental plant fields in Michigan, these nematodes do not need to be included in management decisions.

To determine the impact and threshold levels of *M. hapla* on daylily plants, varying inoculation rates (500, 3,000, 5,000, and 10,000 *M. hapla* eggs/pot) were applied to daylily plants in the greenhouse. The results show that even at the lowest *M. hapla* inoculation rate of 500 eggs/pot, or 13 nematodes/100 cm^3 soil, *M. hapla* can readily reproduce in daylily plant roots and reduce plant growth. This indicates that the threshold value of *M. hapla* is less than 13 nematodes/100 cm^3 soil in these ornamental plant fields. The data also suggests that even though *M. hapla* can adversely affect plant growth, the daylily plants may actually be tolerant to *M. hapla*. Even though significant plant damage was observed at the differing *M. hapla* inoculation levels, at the end of both trials, there was a lack of significantly different plant growth parameters compared to nematode-free plants, suggesting tolerance. This is enforced by the fact that the nematode infested plants did initially grow slower and had a reduction in desirable ornamental plant qualities, such as scapes and flower buds, compared to nematode-free plants, but over the length of the daylily growth cycle, the plants became more tolerant of its feeding and grew to similar sizes of the nematode-free plants. However, the fact that *M. hapla* can still produce galls on daylily plant roots, causing significant economic losses, this pathogen still needs to be properly managed.

Meloidogyne hapla populations are well established in these monoculture, long-term ornamental plant fields. However, when scouting fields for new field trial locations, *M. hapla* population levels were lower than expected, leading to the suggestion of possible soil suppression occurring in these fields. A greenhouse experiment testing the difference between field soil that was fumigated and non-fumigated on *M. hapla* reproduction was conducted, but suggested that soil suppression may actually not be occurring. The non-fumigated soil pots had higher *M. hapla* population levels and daylily plants with lower plant biomass compared to the fumigated soil pots. However, trials investigating natural suppression against *M. hapla* in more than one field need to be conducted. Additionally, testing fields that do not have a history of continual soil fumigation need to also be conducted to determine if natural suppression is actually occurring in ornamental plant fields; this information can then be used to develop even more effective management strategies for *M. hapla*.

Future projects based on the results of this dissertation can include further host status testing of both *M. hapla* and *Paratylenchus* spp. on daylily plants. There are over 98,000 species of daylily, yet only two varieties were screened in this research. Additional host status tests could also be conducted on *M. incognita*, the southern root-knot nematode, which has a larger geographical distribution than *M. hapla*. Determining the efficacy of the top treatments found in this dissertation on *M. incognita* in ornamental plant fields would also provide great value to the ornamental industry and make these results more applicable nationally, especially in the top two ornamental plant production states: Florida and California. Lastly, applying these treatments in other top field-grown perennial ornamental plants, such as hostas and astilbes, would benefit the ornamental plant industry as a whole and provide a much broader impact.

In conclusion, even at the low population level of 13 *M. hapla*/100 cm^3 soil and plant tolerance, *M. hapla* readily reproduces on *Hemerocallis* spp., producing visible galls, which due to ornamental plant inspections, costs the ornamental plant industry huge economic losses. To combat this, this dissertation proposed several effective management strategies that can control the number one pathogen affecting northern North America's ornamental plant fields to prevent this huge profit loss. Additionally, these new management solutions provide the ornamental plant industry with much more sustainable, cost effective, and efficient management options for *M. hapla* compared to current practices, such as chemical fumigation. Therefore, these results will provide an enormous positive impact to the ornamental plant industry, especially since these results can be easily translatable to other ornamental plants. Through using these new alternative management strategies, the ornamental plant industry can become more sustainable and provide significantly better control of *M. hapla* than current management practices thereby reducing the economic loss and exportation rejection this main pathogen causes to the floriculture industry in Michigan, the northern United States, and Canada.

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APPENDIX

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

<u>Voucher Number</u>: 2023-05

<u>Author and Title of Dissertation</u>:
Author: Amanda D. Howland
Title: Determining Alternative and Sustainable Management Strategies to Manage the Northern Root-Knot Nematode (*Meloidogyne hapla*) in Ornamental Plant Production Fields

<u>Museum(s) where deposited</u>:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

<u>Specimens</u>:

Family	Genus-Species	Life Stage	Quantity	Preservation
Heteroderidae	*Meloidogyne hapla*	J2 (2^{nd} stage juvenile)	1	Photograph